CANADIAN JOURNAL OF RESEARCH

VOLUME 19 MAY, 1941 NUMBER 5

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NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

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The Canadian Journal of Research is issued monthly in four sections, as follows:

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Subscription rates, postage paid to any part of the world (effective 1 April, 1939), are as follows:

	Annual	*Single Copy*
A and B	$ 2.50	$ 0.50
C and D	2.50	0.50
Four sections, complete	4.00	—

The Canadian Journal of Research is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. All correspondence should be addressed:

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. C. MAY, 1941 NUMBER 5

THE DEVELOPMENT OF TWIN EMBRYO SACS, EMBRYOS, AND ENDOSPERM IN *POA ARCTICA* R. BR.[1]

BY V. ENGELBERT[2]

Abstract

The origin and development of twin embryo sacs especially in *P. arctica* R. Br. were studied microscopically in sections of whole spikelets representing consecutive stages in development from the time of emergence of the panicle until after pollination. Twin embryo sacs usually develop, one originating from the innermost of a row of four macrospores with the reduced chromosome number, the other from an aposporous cell that originates in the nucellus, near the chalaza and behind the normal archespore. The individual development of the normal and aposporous embryo sacs from their respective mother cells is traced and the competition between them discussed. Both aposporous and normal egg cells develop parthenogenetically. Pollen germination appears to activate the aposporous polar cell to form endosperm which in turn nourishes the aposporous ($2n$) and (or) the "sexual" (n) embryo. The greatest number of plants originate from the aposporous embryo. A type of apospory was found in *P. alpina* L. from Greenland. The work of other investigators, especially on *P. pratensis* L. is reinterpreted in the light of these findings.

Introduction

In a recent publication, the author has made a report of breeding experiments and pollen tube growth studies, the results of which demonstrated apomictic reproduction and pseudogamy in the following species:

P. arctica R. Br. from West Greenland.
P. alpigena Fr. Lindm. from West Greenland.
P. alpina L. from West Greenland.
P. alpina L. from Georgian Bay, Ontario.
P. pratensis L. from Gaspé Peninsula, Quebec.

The present paper deals with those parts of the accompanying embryological studies that seem to the author to provide the information most essential for an interpretation of the *Poa* problem as a whole. This is believed to be the first study of flower biology and embryology of the Greenlandic species or biotypes of *Poa* and the first to trace to their proper origins the two embryo sacs found by many workers in *P. pratensis*, but not as yet explained.

The results in a way fulfil a prediction made by Nannfeldt in 1935 (9) that the *Poa* case parallels Rosenberg's (10) *Hieracium* subgenus *Pilosella*, and suggest a reinterpretation of data supplied in some recent papers dealing with *P. pratensis*.

[1] *Manuscript received December 30, 1940.*
Contribution from the Department of Biology, University of Toronto, Toronto, Ontario.

[2] *Research Assistant.*

Material and Methods

Culture

Seeds of *P. arctica* harvested in Greenland were used to raise the original plants. These seeds, which were chilled before seeding, had a lower percentage germination than those of the other species. To grow this high arctic species here at approximately 43½° N. latitude and 300 to 400 ft. above sea level was found very difficult. Certain conditions had to be provided to keep the plants alive and sufficient material at hand for experiments. The species is narrowly restricted ecologically and does not withstand either desiccation or water surplus as does *P. alpigena*. It takes two years to raise *P. arctica* to flowering and to get suitable experimental material of the other species. Flowering occurred at the ordinary length of day found here in April and May, but it was found that *P. arctica* cannot be kept alive after flowering without being given a longer day. Continuous daylight was provided by a 60-Watt electric bulb suspended 28 in. above the plants. This produced good vegetative growth when other conditions were adjusted to keep a proper water balance and to eliminate the effect of high temperature.

Technique

Whole spikelets or whole panicles were fixed from two to several times a day from the time the top of the panicle began to emerge to several days after anthesis. Fixations were made by immersion for 1 min. in Carnoy's fluid then in La Cour's* 2BE for 24 hr.; later La Cour's 2BD was used as it seemed to give somewhat better results. Sections were cut 8 μ thick.

Staining was at first done with crystal violet, later with the Feulgen stain as formulated by Dr. L. C. Coleman**; the time of hydrolysis used was the one determined by Dr. B. B. Hillary***. Photomicrographs were made with a Leica camera attached to a Spencer microscope. An apochromatic 2 mm. (*N.A.* 1·30) oil immersion objective (90 ×) and a Leitz ocular (10 ×) were used for Plate I (*A*, *B*, *C*, *D*, and *F*). For Plate I-*E*, a dry 60 × objective was used. Illumination was provided by a lamp designed, by Dr. D. H. Hamly†, after the Koehler arrangement. A combination of orange and green filters was used.

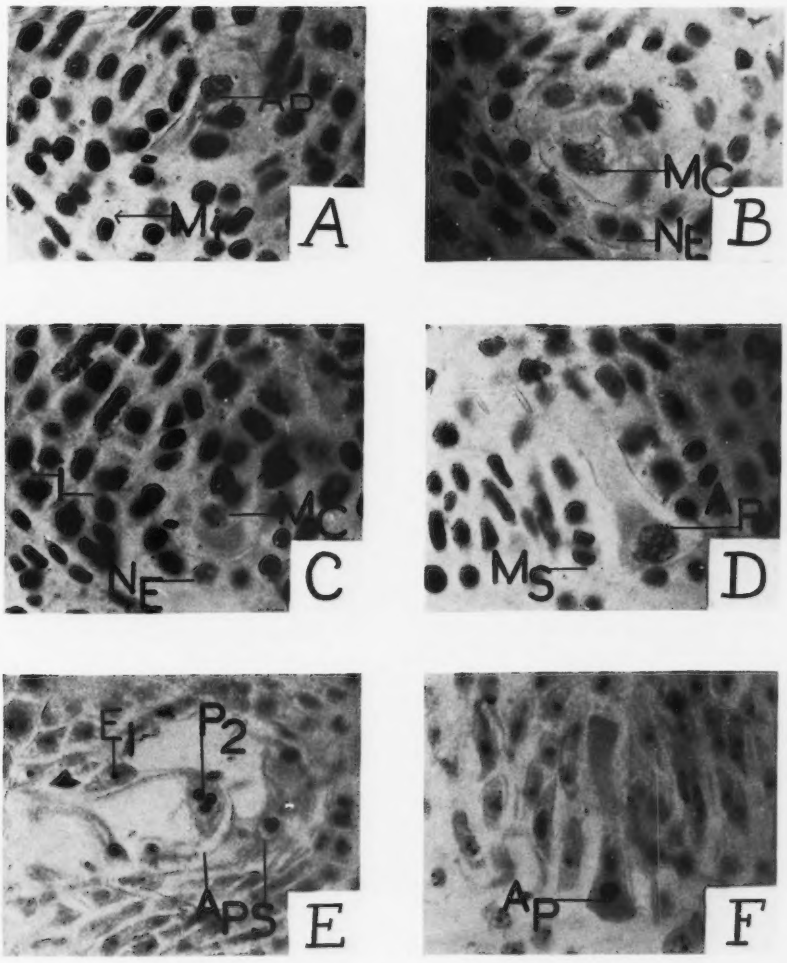
Embryology

Development of Twin Embryo Sacs

Origin of the two embryo sac mother cells

In the earliest stage the normal archespore (M_C, Fig. 1-*A*) is found just below the nucellar epithelium. The somatic, aposporous cell (A_P) is distinguishable at the same time behind the archespore near the chalaza.

* *LaCour, L. A. J. Roy. Micr. Soc. 51 : 119-126. 1931.*
** *In laboratory outline for cytology, Department of Botany, University of Toronto.*
*** *Hillary, B. B. Bot. Gaz. 10(2) : 276-300. 1939.*
† *Department of Botany and the School of Practical Science, University of Toronto.*



Photomicrographs of longitudinal sections of young ovules in different embryonical stages. Figs. A, B, C, D, and E, Poa arctica. Fig. F, Poa alpina. Figs. A and C are from the same nucellus. See legend under Fig. 1.

Development of the normal type embryo sac from the normal archespore

The normal embryo sac develops from the fourth and innermost of four macrospores formed by two meiotic divisions from the normal archespore. The development is diagrammatically shown in Fig. 1. The normal archespore is first found just below the nucellar epithelium (Fig. 1-*A* and Plate I-*C*). A later stage showing the nucleus in prophase is shown in Plate I-*B*. The archespore occupies a large part of the young nucellus and, the nucleus especially, undergoes considerable growth before the meiotic division. Two daughter cells with the reduced chromosome number are formed (Fig. 1-*B*) and these give rise to four macrospores (Fig. 1-*C* and Plate I-*D*). The three outermost of these degenerate. (In Plate I-*D* the outer two have begun degeneration.) The fourth and innermost undergoes a period of growth and of vacuolization (sap-uptake) in preparation for the first embryo sac nuclear division. The fourth macrospore in Plate I-*D* has already increased in size (its nucleus is somewhat out of focus in the photograph). Not all the nuclear divisions in the embryo sac have been followed but young embryo

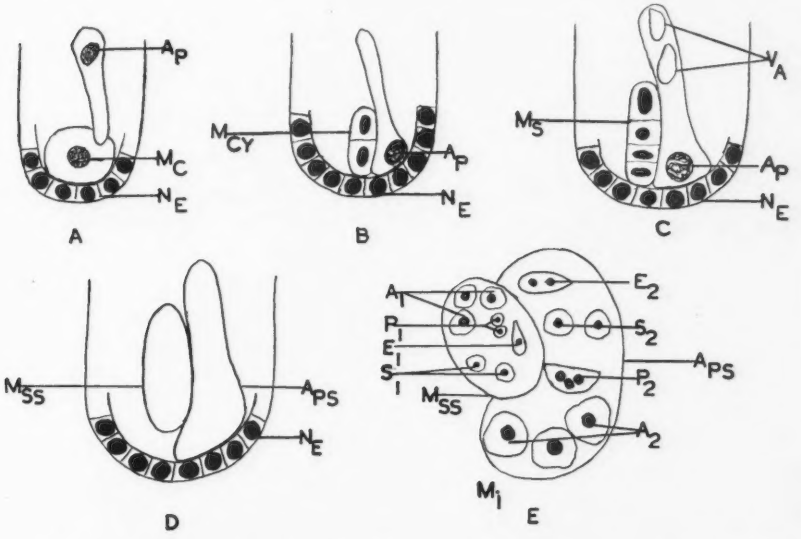


FIG. 1. *Normal embryo sac development accompanied by apospory as found in P. arctica R. Br. from Greenland, diagrammatically illustrated.* A_P, *aposporous cell;* A_{PS}, *aposporous embryo sac; A, antipodals; E, egg cell; P, polar cell; S, synergids;* M_{SS}, *macrospore embryo sac;* M_i, *micropyle;* M_C, *macrospore mother cell (normal archespore);* M_S, *macrospores;* N_E, *nucellar epithelium;* M_{CY}, *macrosporocytes; two archespore daughter cells;* V_A, *vacuoles. A. Normal archespore below nucellar epithelium, elongating aposporous cell behind it. B. Two archespore daughter cells. The aposporous cell has reached the micropyle and its nucleus has wandered out into the tip. C. Four macrospores. The nucleus of the aposporous cell in prophase before first embryo sac division. D. Two young twin embryo sacs. E. Two fully developed twin embryo sacs. Constructed lateral view of the twin embryo sacs photographed in Plate I-E.*

sacs with four and six nuclei respectively have been observed and also several fully developed eight-celled embryo sacs. Although they are smaller than the twin (aposporous) embryo sac and somewhat crowded by it, each contains a uninucleate egg cell, two uninucleate unfused polar cells, three antipodals, and two synergids (Fig. 2-*E* and Plate I-*E*).

Development of a somatic aposporous cell into the second embryo sac

The earliest stage of development of the somatic aposporous cell is distinguishable at the same time as the earliest stage of the normal archespore and is shown in Fig. 1-*A* and Plate I-*A*. *A* and *C* (Plate I) are photographs of sections of the same nucellus only 8 μ apart. (These are diagrammatically shown in Fig. 1.) It will be noticed that the aposporous cell that lies near the chalaza behind the archespore first stretches in the direction of the micropyle (Fig. 1-*A*). When its tip reaches the nucellar epithelium it flattens out somewhat and the nucleus which has wandered from its original position reaches the cell tip at about the time that the archespore has developed into two daughter cells (Fig. 1-*B*). The aposporous cell now undergoes further growth which involves a great sap-intake (see vacuoles, Fig. 1-*C*). These cannot be seen in the photograph (Plate I-*D*) but could be easily seen when the section was first mounted and stained.

Some increase in the size of the nucleus is evident and the aposporous cell is now ready for its first embryo sac division. The nuclear divisions of this embryo sac are not at present completely understood but they appear to be mitotic.

This embryo sac is seven-celled (Fig. 1-*E*). The three antipodals are large and two synergids are also present.

Competition of the two embryo sacs

The competition between the two embryo sacs for nutrition and space must mainly depend on the fact that the aposporous embryo sac, because of its origin near the chalaza (and thus the vascular system), has an immediate advantage. Factors influencing rate of growth of both embryo sacs must next be considered, that is, whether these are genetic or connected with nutrition and water-balance of the ovule as conditioned by these two factors for the whole plant.

It seems that the normal embryo sac survives surprisingly well and the author believes that the usual twin embryo sac combination is one normal and one aposporous. Death of either the normal or aposporous embryo must be expected in some ovules leaving the other embryo sac the full space.

Development of Embryos and Endosperm

Parthenogenetic development of embryos

Parthenogenesis of the normal egg cell

Embryological evidence is not yet clear for *P. arctica* but the occurrence of weak twin and single seedlings, much less vigorous, smaller, and with much narrower leaves than the sister seedlings seems to point also to parthenogenetic

development of the normal egg. Twins, one haploid, one diploid are believed to be found in *P. alpigena*.* The evidence for parthenogenesis of the normal egg in *P. pratensis* and *P. compressa* seems very convincing especially as interpreted from the careful observations of Miss Andersen (3)** and also from Tinney (11). Although none of the investigators who have shown the embryological evidence in their illustrations reports it*, Müntzing (8) shows that two *P. pratensis* plants that obviously are twins have 36 and 72 chromosomes respectively but he could not, at the time, give the embryological explanation.*

Parthenogenesis of the aposporous egg cell

That the aposporous egg cell develops parthenogenetically is indicated by the fact that it is multicellular before anthesis or pollination, and emasculated, unpollinated flowers dissected at harvest time can show development of embryos. Since there is no endosperm, however, there is no seed formation. Further, inhibited pollen tube growth in the stigma shows that fertilization is not the rule (5) and this, combined with the matroclinic appearance of all the plants raised from cross pollinations (5) shows that these originated from the aposporous ($2n$) egg cell. That the aposporous embryo develops by parthenogenesis in *P. pratensis* and *P. compressa* is evident from Miss Andersen's work (3) although she could not interpret it at the time. Tinney (11) states for *P. pratensis*, "the diploid egg develops into a proembryo by parthenogenesis. The development frequently begins before pollination."

Role of pollination in endosperm development (pseudogamy)

As already mentioned, the endosperm was not developed in emasculated, non-pollinated flowers although embryos were found. Pollination was thus found necessary for development of a complete seed in *P. arctica*, *P. alpigena*, and in *P. alpina* as well as in *P. pratensis* (5).

Aakerberg (1) reported that no seed was produced in *P. pratensis* in emasculated, unpollinated flowers and suggested pseudogamy as necessary for seed production.

Observations by the author of pollen germination and pollen tube growth from 2 to 36 hr. after pollination on the above *Poa* species showed that the ovary had increased in size at that time but the pollen tubes remained short. These facts combined with the embryological data made it evident that the germination of the pollen on the stigmas stimulated the development of the aposporous polar cell to an endosperm (pseudogamy).

Miss Andersen's data show clear evidence of this phenomenon in both *P. pratensis* and *P. compressa*, although she could not interpret this at the time. Her Plate 7-*B* shows two embryos (the "sexual" (?) and the aposporous ($2n$)) close to a large developing endosperm above which are the two nuclei of the fused but undeveloped polar cells of the normal embryo sac.

* *See section on Reinterpretation of some other papers on Poa.*
** *See Plate 7-B and Plate 8-C in (3).*

Tinney (11) suggests for *P. pratensis* that "since endosperm development was not observed to begin until after pollination, it may be that pollination or the growth of pollen tubes in stylar tissue is necessary for endosperm development and consequently for seed development. Pollen tubes have not been observed in the embryo sac." In Andersen's Plate 8-*C* (3), the scutellum of the larger, aposporous embryo lies pressed in against the endosperm whereas the smaller embryo does not reach the endosperm at all.

Tinney (11) shows in his Plate 3-*H* and Plate 4-*B*, two embryos almost imbedded in the large endosperm. In Plate 3-*H* the polar cell of the normal embryo sac lies undeveloped.* Tinney does not believe the normal embryo sac develops.

Andersen's Plate 7-*B* and Tinney's Plate 3-*H* and also Andersen's Plate 8-*C* and Tinney's Plate 4-*B* compare well.

Development of Twin Seedlings

Parthenogenetic development of the normal egg cell is believed by the author to accompany parthenogenesis of the aposporous egg cell.

Twin pairs of plants, one member showing matroclinic ($2n$) characters, the other showing aberrant characters have been obtained recently in numbers up to 5% in *P. alpigena* and *P. pratensis*.

Twins, both of which show matroclinic ($2n$) characters, have been found in one half of one per cent of germinated seeds in *P. alpigena* and in *P. pratensis* and in larger proportions of *P. arctica* and *P. alpina*.

A paper dealing with these twin plants and their origin as well as the method used in raising them is being prepared.

The author believes that if both the "sexual" (n) and the aposporous ($2n$) embryos are in favourable positions in relation to the mass of the aposporous endosperm then this can nourish both of them to germination.

The scutellum of the *Poa* seed is a shield-like organ of absorption (3) through which the growing parts of the embryo receive their nutriment from the endosperm during germination. Andersen (3) shows stages of development of the embryos of *P. pratensis* and *P. compressa* and the extension of the scutellum. The author believes that only in the rare instances of actual fertilization by the pollen will the polar cells of the normal embryo sac develop into an endosperm. Fusion of the two cells may happen after pollination.

It seems evident that the aposporous embryo (and endosperm) give rise to the usual and most common plants, hence the matroclinic appearance of such great proportions of progeny (5, 12). Further, one cause of variation in progeny must be the fact that the "sexual" (n) embryo develops and produces a seedling because it is in a sufficiently favourable position in relation to the

* *See section on Reinterpretation of some other papers on Poa.*

aposporous endosperm to be nourished by it and also because of rare instances of fertilization. As they seem morphologically similar to the haploid member of twin seedlings many non-viable or weak seedlings encountered in greenhouse work may be from haploid embryos. There may be a slower or lesser scutellum development in this seed than in the aposporous one. If, of twin plants, one is diploid and one triploid as reported by Müntzing (7) then the possibility of their origin may be as follows: the diploid plant may have arisen from a fertilized normal egg cell and the triploid plant from a fertilized aposporous egg cell. Fertilization can occur in rare cases. Possibilities of development of cells other than egg cells into embryos (apogamety) will be considered in a later paper.

Reinterpretation of Some Other Papers on *Poa* in the Light of the Present Findings

The development of four macrospores from the normal archespore was reported by Andersen (3) and Armstrong (4). A normal archespore, but according to his illustration a very different form from the ones found by the author and others, was reported recently by Aakerberg (2). Tinney (11) reports "a single, elongated, very conspicuous macrospore mother cell with the nucleus located usually near the micropylar end, or in some instances near the chalazal end". This shows clearly that he has confused the aposporous cell with the normal archespore cell. He believes three or four macrospores are formed but that they all die. Aamodt (12) believes "that there is no apparent reason why an occasional macrospore should be prevented from functioning and, if an embryo sac were formed, the haploid egg might either produce an embryo by parthenogenesis or develop into an embryo following fertilization."

Andersen (3) and Armstrong (4) believe that two macrospores can develop into embryo sacs. Aakerberg (2) and Tinney (11) are of the opinion that all macrospores die.

Andersen and Armstrong both confuse the nucleated tip of the aposporous cell with the "macrospore" near the micropyle that "sometimes" develops. Armstrong's Fig. 33 shows the aposporous cell cut in half.

Aakerberg (2) reports an aposporous cell but his illustration shows a type of undifferentiated cell found in numbers in the chalazal part of young nucelli before the aposporous cell shows up; he could not have seen the characteristic elongation of this cell.

Tinney (11) fully recognizes apospory (he finds approximately the diploid chromosome number in young embryo sacs). But he shows the fourth macrospore in the vacuolization stage and believes it to be "a somatic cell differentiated from the nucellus" and "destined to function as the initial cell of the embryo sac." He shows in Plate I-*C* the fourth macrospore with a large vacuole, and a smaller aposporous cell next to it that is just beginning to stretch. He believes these two cells to be the two twin embryo sac

primordia. In the same illustration he shows the fully developed aposporous cell which he believes is the normal archespore.

It is commonly agreed by all workers that polyembryony is very frequent, but the origin of the twin embryo sac has been the disputed point. In Table I the author has outlined the theories of the various workers and her own conclusions.

TABLE I

REPORTS OF VARIOUS AUTHORS CONCERNING THE OCCURRENCE AND ORIGIN OF EMBRYO SACS AND SEEDLINGS

Author	Twin embryo sacs			Twin plants			
	Occurrence	Origin		Occurrence	Origin		
		Normal	Aposporous		Haploid (reduced)	Diploid (unreduced)	Triploid
Andersen	Yes	Two	—	—	—	—	—
Armstrong	Yes	Two	—	—	—	—	—
Aakerberg	—	—	—	Yes	Reduced	Unreduced	—
Tinney	Yes	—	Two	—	—	—	—
Müntzing	—	—	—	Yes	Haploid (36)	Diploid (72)	Triploid
Author	Yes	One	One	Yes	Haploid	Diploid Both diploid (matroclinic)	—

Apomixis (parthenogenesis) and pseudogamy have been recognized in *Poa* since Müntzing (7) and Aakerberg (1) established knowledge of these facts, but it is only recently that Tinney (11) and the author have suggested new theories that explain the details of the mechanism of these phenomena.

Tables II and III show the early and new theories tabulated according to author and seniority and sequence of findings.

From the evidence given in this paper and an interpretation of the literature, the author feels that the twin embryo sacs so commonly found in the polyploid, polymorphous *Poa* species are, as a rule, developed one from a macrospore, the other from an aposporous cell.

It should be emphasized that clear evidence is found of both the development of the normal and the aposporous embryo sac especially in Andersen's, and also Armstrong's figures, and in descriptions and illustrations in Tinney's (11) work. The latter mentions in his paper "the smaller embryo sac" and the "unfused polar cells" which belong to it, also endosperm cells with four and five nuclei (here interpreted as belonging to aposporous embryo sac). He mentions twin embryo sacs where "there were five polar nuclei in one embryo sac and two in a companion sac."

To explain the aberrant types appearing in his progeny test of *P. pratensis*, Tinney and Aamodt (12) had to postulate the development of the occasional macrospore. There seems to be a difference between biotypes in *P. alpina*

TABLE II

APOMIXIS (PARTHENOGENESIS) AND PSEUDOGAMY AS REPORTED IN THE GENUS *Poa*

	Author					
	Müntzing 1932	Kiellander 1935	Aakerberg 1936	Flovik 1937	Tinney 1940	Present author 1940
	Species					
	P. alpina *P. pratensis*	*P. serotina*	*P. alpina* *P. pratensis*	*P. arctica* *P. alpigena*	*P. pratensis*	*P. arctica* *P. alpina* *P. alpigena* *P. pratensis*
Apomictic seed production reported on basis of:	Cytology and breeding experiments	Cytology	Breeding experiments	Cytology	—	Breeding experiments
Necessity of pollination for seed production and activation of embryo sac. Pseudogamy reported on basis of:	—	—	Pollination experiments	—	—	*(a)* Pollination experiments *(b)* Pollen tube growth studies

TABLE III

PARTHENOGENETIC DEVELOPMENT OF EMBRYOS AND ENDOSPERM

	Author		
	Tinney 1940	Present author 1940	
	Species		
	P. pratensis	*P. arctica* *P. alpigena*	*P. alpina* *P. pratensis*
I. Parthenogenesis of unreduced aposporous egg cell before pollination reported on basis of:	Embryological observations	*(a)* Matroclinic appearance of progeny *(b)* Embryological observations (*P. arctica*) *(c)* Embryos in unpollinated flowers *(d)* Interpretation of literature of *P. pratensis* and *P. compressa*	
II. Parthenogenesis of normal egg cell reported on basis of:		*(a)* Twins in *P. alpigena* and *P. pratensis* *(b)* Interpretation of literature on *P. pratensis* and *P. compressa*	
III. Endosperm developpment of aposporous polar cell by pseudogamy *after pollination* reported on basis of:	Embryological observation before and after pollination	*(a)* Observation of lack of endosperm in unpollinated flowers *(b)* Interpretation of literature on *P. pratensis* and *P. compressa*	

and *P. pratensis* in "sexuality" as reported by Müntzing (8) and Aakerberg (2). This difference may be based on the fact that the normal egg cell develops more often in some strains than others.

It should be borne in mind that the ability of the haploid embryo to survive in *P. pratensis* and *P. compressa* may vary from one strain to another, and certain strains may therefore show a higher percentage of aberrants or higher percentage of twins than others.

Aakerberg (2) reports that 11.2% of the plants in an "apomictic" strain were twins and that 2.9% were twins in a "sexual" strain. The fact that there are twins at all in the "sexual" strain points to apospory being present here also, although Aakerberg claims not to have found aposporous embryo sac mother cells in this strain.

Plant breeding selection work with *P. pratensis* and *P. compressa* could be facilitated considerably if an initial embryological and seed germination investigation were made to determine the degree and type of twinning and thereby the number of aberrants to be expected.

Further work on the pairs of aberrant and matroclinic twin plants as well as on the pairs of matroclinic twin plants will be compiled for publication.

Acknowledgments

The author is greatly indebted to Dr. O. McConkey for suggesting the *Poa* problem and for suggesting the importation of seed from Greenland; to Magister M. P. Porsild, Director of the Danish Arctic Station, Disko Island, Greenland, for personally collecting and sending the *Poa* seed and for his valuable advice; to Dr. J. W. MacArthur for direction, advice, and never failing interest in the development of the problem.

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STERILITY IN POTATOES[1]

By T. J. Arnason[2]

Abstract

Microspore tetrads were rarely formed in three male-sterile potato varieties studied. Dyads and triads were much more common. The failure of the second meiotic divisions appeared to be the main reason for the lack of tetrads. Many of the microspores aborted without enlarging, some did grow, however, and round off. In presumably mature anthers from open flowers, microspores were very variable in size and in the appearance of the nucleus and cytoplasm. Anther pores usually failed to open. The pollen-fertile varieties formed microspores that were almost entirely in tetrads. Mature anthers had roomy locules, open anther pores, and large numbers of uniform, sound-appearing pollen grains. They contained also some empty grains. About one-half the grains from one pollen-fertile line were empty. Abscission of buds and flowers is an important factor contributing to sterility or at least unfruitfulness in many potato varieties, including the three male-sterile ones reported in this paper. Following pollination with sound pollen, a few seeds have been obtained, though with some difficulty, from each of the male-sterile lines. Premature flower abscission rather than female sterility appears to be the main bar to seed production when sound pollen is applied.

Introduction

Breeding programs designed to discover the genetics of potato characters and those designed to produce superior new lines are often hampered by sterility. Many of the varieties most productive of good tubers ordinarily do not set seed. Selection for superior tubers may involve selection of non-fertile plants since in fertile plants more carbohydrate must be retained in the fruiting tops of the plants than in unfertile ones. With advances in knowledge of sterility in potatoes considerable saving of effort in breeding for ideal commercial types may be effected. That new varieties are needed is evidenced, for instance, by the fact that in Western Canada no early maturing, virus resistant, scab resistant, shallow eyed, well shaped potato of good cooking quality exists.

Each of the commercial varieties of potatoes has its own combination of characters, good and bad. The incorporation of the good characters of different clones in individual derivatives is difficult because of the sterility of most of the commercial varieties. It is possible that when the reasons for sterility are known, methods of overcoming it may be found in some cases at least.

A number of workers have investigated sterility in potatoes. It has been shown that failure to set seed may be attributed to bud abscission, meiotic irregularities in anthers, abortion of microspores, and abortion of embryo sacs. Stout and Clark (4) have supplied evidence to show that certain potato varieties may shed all their buds under certain conditions, form many flowers

[1] *Manuscript received February 6, 1941.*
Contribution from the Department of Biology, University of Saskatchewan, Saskatoon, Sask.

[2] *Assistant Professor of Biology.*

under other conditions. Sterility in which early abscission is the chief cause might be overcome by keeping plants cool when crosses were desired. The evidence (4) indicates that relatively low temperatures favour the retention of buds and flowers.

Failure of the meiotic divisions in anthers has been observed by a number of investigators (1, 2, and 6). According to Stow (5) meiotic failure is to be attributed to high temperatures. An experiment conducted by Ellison (1) failed to confirm this conclusion. Genetic as well as environmental factors must be taken into consideration since different varieties grown under similar conditions differ with respect to amount and length of flowering, regularity of meiotic divisions and pollen and seed production. Female sterility is much less marked than male sterility. Rees-Leonard (3) has reported, however, some evidence of embryo sac abortion in the variety Irish Cobbler.

Materials and Methods

Twenty lines of potatoes have been kept under observation for four seasons. Of these 15 were numbered seedling lines obtained from Dr. G. Rieman of the University of Wisconsin and five were commercial varieties kindly supplied by Dr. C. F. Patterson of the University of Saskatchewan. Three sterile or unfertile kinds were selected for special study. These were the United States Department of Agriculture selections 46000 and 44488 (Sebago) and the variety Early Ohio. Five lines were highly pollen-fertile. These were the U.S.D.A. selections 46422 and 45075 (Earlaine), Minnesota selections 11-1-2-1, 82-11, and 75-5. Observations were made on bud, flower, and fruit abscission, quality and quantity of pollen, and on meiotic divisions in anthers.

Pollen from ripe anthers was studied in aceto-carmine mounts. Meiotic divisions were studied in aceto-carmine smear preparations and in permanent sections. Material to be sectioned was fixed for 1 min. in Carnoy's fluid (3 parts absolute alcohol : 1 part glacial acetic acid) then in Karpechenko's modification of Navashin's fixative for 12–24 hr. The anthers were imbedded in Parlax which is superior to ordinary wax. The sections were cut at thicknesses of 12 to 16 μ and stained in Delafield's haemotoxylin for 1 min. before passing through crystal violet and iodine solutions.

Bud Abscission and Length of Flowering Period

Failure of potatoes to set seed is not always owing to pollen or embryo sac sterility. The abscission of buds, flowers, and young fruits effectively prevents the formation, development, or ripening of seeds in many cases. The proportion of buds and flowers that drop off varies between varieties and also within varieties grown in different seasons or under different conditions.

In two consecutive seasons all the buds of Early Ohio plants dropped off before the flowers opened. In the third season after a particularly cold and wet June many flowers opened in the first week of July, but later buds, formed and developed in hot dry weather, all dropped off. The U.S.D.A. 46000

line behaved similarly except that a much larger number of buds were formed and a few of the buds developed into open flowers each season. Quite commonly there were 15 or more buds in a young cluster. Seldom did more than 10 of these reach the open flower stage, often the number was much lower. When flowers opened they had a tendency to persist for several days at least. Plants of the U.S.D.A. 44488 (Sebago) line formed fewer buds than did U.S.D.A. 46000. Most of the buds dropped off in each season but single open flowers here and there showed that the abscission mechanism did not cut off all buds before flowering. However, the open flowers almost invariably fell off within a few days.

Flowers of the U.S.D.A. 45075 (Earlaine) pollen-fertile variety were formed in abundance in all four seasons. Clusters of seven or more flowers were common. Even so, however, there was always some bud abscission. The length of the flowering period of this and other pollen-fertile varieties varied (Table I) from season to season indicating the importance of undetermined environmental factors. In some of the fertile varieties, buds were formed usually over a period of several weeks. The flowering period would undoubtedly be considerably longer for most varieties if there were not abscission of all buds during a part of the season.

TABLE I

LENGTH OF FLOWERING PERIOD OF SEVERAL VARIETIES OF POTATOES GROWN AT SASKATOON[1]

Variety	1937	1938	1939	1940
U.S.D.A. 46000	July 24 – Aug. 19	July 5 – 12	July 7 – 15	June 28 – Aug. 5
U.S.D.A. 44488	July 25 – Aug. 19	July 16 – 29	July 2 – 19	June 21 – Aug. 5
U.S.D.A. 45075	June 23 – Aug. 17	July 5 – 14	June 29 – July 15	June 17 – July 22
Minn. 11–1–2–1	July 30 – Aug. 15	July 14 – 30	July 6 – Aug. 15	July 2 – 29
Minn. 82–11	July 15 – Aug. 19	July 5 – Aug. 2	July 2 – 15	June 24 – July 29
Minn. 75–5	July 1 – Aug. 5	July 12 – 30	July 5 – Aug. 15	June 28 – July 29
Irish Cobbler	Not recorded	? – July 23 – ?	June 29 – July 25	June 24 – July 15
Early Ohio	No flowers	No flowers	July 2 – 10	No flowers

[1] *The pedigree numbers and names are those supplied with the original samples of tubers.*

Mature Pollen

When ripe anthers of pollen-fertile potatoes are tapped sharply on a hard object, such as a thumb nail, easily seen dry yellow pollen is sifted through the subterminal pores and deposited. Although numerous trials were made, especially with the U.S.D.A. 46000 line, U.S.D.A. 46000 and Early Ohio anthers did not yield any pollen. In Table II are given the average percentages of sound-appearing grains in many pollen samples examined. In all varieties there was considerable variation in the proportion of good grains. Consequently the percentages given should be considered as no more than generally indicative of soundness. A point not brought out by the table is the fact that samples taken from any productive variety at different times in the same season show considerable differences.

TABLE II

NUMBER OF SOUND-APPEARING GRAINS IN POLLEN SAMPLES (AVERAGE PERCENTAGES)

Variety	1937	1938	1939	1940
U.S.D.A. 46000	No pollen	No pollen	No pollen	No pollen
U.S.D.A. 44488	13.3*	17.8*	No pollen	1.0*
U.S.D.A. 46422	61.5	55.7	41.9	39.0
U.S.D.A. 45075	16	72.6	60.9	58.8
Minn. 11–1–2–1	84.7	93.4	94.0	79.0
Minn. 82–11	64.6	89.8	56.9	75.8
Minn. 75–5	53.0	76.8	82.5	60.0
Early Ohio	No flowers	No flowers	No pollen	No flowers

* *Pollen very scanty.*

NOTE: *The soundness of the pollen of U.S.D.A. 45075, Minn. 11–1–2–1, 82–11, and 75–5 has also been proved by successful crosses.*

Meiotic Divisions

A small proportion of cells of both pollen-fertile and pollen-sterile varieties showed slight irregularities in the first meiotic division. In both, however, bivalents at metaphase and two compact chromosome groups at late anaphase were the rule. It is mainly at the conclusion of this division that the pollen-sterile lines begin to show noticeable peculiarities in nuclear behaviour.

In the pollen-fertile U.S.D.A. 46422 and 45075, the second division follows the first rather closely. The two spindles of the second division are parallel in some cells, in others at right angles to each other. Counts of cells of U.S.D.A. 46422 gave 51 with spindles parallel or nearly so, 27 with the spindles at right angles. In these varieties wall formation begins shortly after the

second nuclear division has taken place. Spore tetrads occur in abundance. Malformed or abnormal cells are rare or absent.

Deviations from the "normal" development of the pollen-fertile lines become marked after the first division in all three of the pollen-sterile lines examined. The cause of the breakdown at this stage has not been determined. Since some differences between the cells of the sterile varieties were observed they will be described separately.

U.S.D.A. 44488

Twenty-four chromosomes were counted in several polar views of first division anaphases. As in the fertile material, no walls were formed at the end of this division. The two nuclei moved to near the centre of the cell, enlarging considerably before the nuclear membranes disappeared. At the second division metaphases, the chromosomes of the two nuclei sometimes were grouped together on a single plate (Fig. 12). Frequently two metaphase plates were visible however, but the orientation of these was often highly irregular, e.g., the two spindles converged on one side or the spindles were crossed so that at anaphases some chromosomes were apparently stranded in the mid-region (Fig. 14). In late anaphases the chromosomes were in two, three, or four groups. Single chromosomes sometimes failed to be incorporated in the telophase nuclei. The number of normal appearing spore tetrads formed was small. A count of the number of cells in 100 cells or groups derived from single pollen mother cells gave the following results:

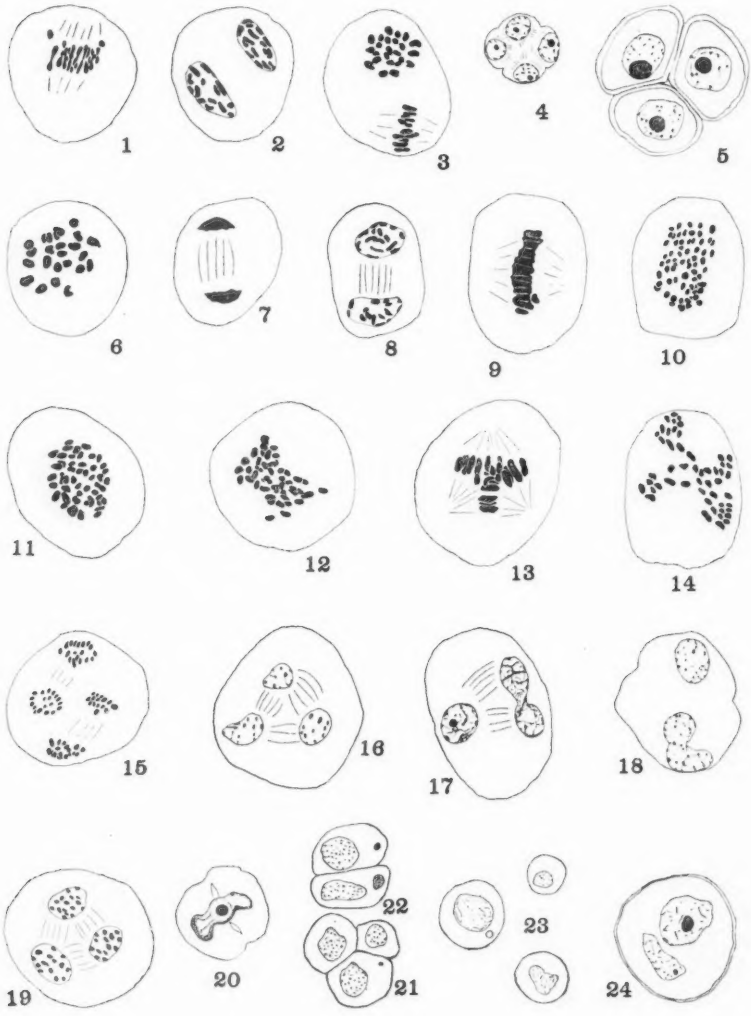
4 cells	(tetrads) approximately equal in size	7 groups
4 cells	markedly unequal	11 groups
3 cells	unequal in size	41 groups
2 cells	some equal, some not equal	40 groups
1 cell		1
		100

In the dyads and triads densely staining granules (Figs. 21, 22) were visible in about 25% of the cells. These were probably chromosomes that had failed to be included in the nuclei. Lobed nuclei, formed by fusion of more or less distinct chromosome groups, were common.

U.S.D.A. 46000

The first meiotic division was slightly more irregular in this than in other lines. Even so, the majority of late anaphase figures showed two compact chromosome groups. A count of 84 cells gave 80 with no visible irregularity in late anaphases, three cells with one or two lagging chromosomes, and one cell with a thin chromatin strand connecting the two chromosome groups. On the whole there was little visible irregularity until after the first division telophases. Following the telophase stages the nuclei moved toward the centre of the cell becoming somewhat pycnotic and shrinking in size. Few second division figures could be found, probably because they failed to occur.

In a large proportion of second division equatorial plate stages the chromosomes of the two nuclei were spread on one plate (Fig. 11). A single bipolar spindle was most commonly observed. In some cases the single plate was L- or T-shaped or otherwise irregular. Some of the crossed plates were observed to have tripolar spindles (Fig. 13).



FIGS. 1–24.

Few anaphases of the second division were seen. The telophase nuclei were, as might be expected, two in most cells, three in a small proportion. The three were doubtless produced by the functioning of tripolar spindles. A count of 200 cells at this stage showed 24 uninucleate, 146 binucleate, 27 trinucleate; three cells had two nuclei and a small chromatic mass or micronucleus. The uninucleate condition may be more common at a later stage as it looks as though two nuclei sometimes fuse. The uninucleate condition may probably be caused also by failure of nuclear division after the fusion of first division nuclei. In many of the cells after the attempted second division the nuclei are small and highly pycnotic. This is interpreted as a sign of nuclear breakdown. It is not certain that two divisions invariably take place in pollen mother cells. In any case when wall formation takes place there are most commonly two, sometimes three nuclei present. Dyads are most abundantly formed. Even when there is but one large, lobed nucleus a partition wall sometimes begins to form (Fig. 20). When there are three distinct nuclei, three distinct cells may form.

Early Ohio

Most figures of the first division appeared regular but were much delayed in many cells. Smears of anthers containing second division metaphase figures also showed nuclei in late diakinesis and many stages intermediate between these two. Side views of second division metaphases often bore a superficial resemblance to first division metaphases (Fig. 9). This was interpreted as being caused by fusion of first division nuclei and the alignment of their chromosomes on a single plate. In polar view this plate was sometimes

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EXPLANATION OF FIGURES

For all figures, 10× and 20× compensating oculars were used. For FIG. 20, *a Spencer 4 mm. objective of N. A. 0.85 was employed; for all other figures a Zeiss oil immersion apochromat objective of N. A. 1.4. Drawings were made at table level with the aid of a Spencer Abbé camera lucida. All figures have been reduced one-half in reproduction.*

FIGS. 1 TO 5 *are of cells from anthers of pollen-fertile lines. All others are from pollen-sterile lines.*

FIG. 1. *U.S.D.A. 46422. Heterotypic division. 1000×.* FIG. 2. *Minn. 75–5. Interphase. 1000×.* FIG. 3. *U.S.D.A. 46422. Homoeotypic metaphase. 1000×.* FIG. 4. *Minn. 11-1-2-1. Young tetrad. 540×.* FIG. 5. *Minn. 75–5. Three of the four cells of an older tetrad. 1000×.* FIG. 6. *U.S.D.A. 44488. Heterotypic division. 1000×.* FIG. 7. *Early Ohio. Late anaphase I. 1000×.* FIG. 8. *Early Ohio. Interphase I. 1000×.* FIG. 9. *Early Ohio. Metaphase II. Single plate. Side view. 1000×.* FIG. 10. *Early Ohio. Metaphase II. Single plate. 1000×.* FIG. 11. *U.S.D.A. 46000. Metaphase II. Single plate. 1080×.* FIG. 12. *U.S.D.A. 44488, Metaphase II. Single plate. 1000×.* FIG. 13. *U.S.D.A. 46000. Metaphase II. Plates crossed. Tripolar spindle. 1000×.* FIG. 14. *U.S.D.A. 44488. Anaphase II. Spindles crossed. Many chromosomes stranded. 1000×.* FIG. 15. *Early Ohio. Anaphase II. Four separate groups. A rare occurrence. 1000×.* FIG. 16. *U.S.D.A. 46000. Three nuclei formed at the end of division II. 1000×.* FIGS. 17, 18. *U.S.D.A. 46000. Three nuclei formed at the end of division II. Two of the nuclei fusing. 1000×.* FIG. 19. *Early Ohio. Three nuclei formed at the end of division II. 1000×.* FIG. 20. *U.S.D.A. 46000. Wall apparently about to cut through large single nucleus. 665×.* FIG. 21. *U.S.D.A. 44488. Microspore triad formed after division II. Micronuclei. 540 ×.* FIG. 22. *U.S.D.A. 44488. Microspore dyad. 540×.* FIG. 23. *U.S.D.A. 44488. Group of three young pollen grains of different sizes. All from the same anther. 540×.* FIG. 24. *U.S.D.A. 44488. Microspore from mature anther, two nuclei. 1000×.*

round, to be expected if nuclear fusion preceded the formation of the plate. More often the plate appeared as two overlapping circles, an appearance to be expected if the nuclei were very close together or only slightly fused at the time of the disappearance of the nuclear membranes (Fig. 10). In side view the second division plate was narrower and had smoother margins than first division plates. The individual chromosomes were smaller and, of course, more numerous. Some of the second division plates as observed in side view were curved or bent in L, V, or T shape. These had tripolar spindles.

In a few cells two distinct second division metaphase plates were seen (Fig. 15). At later stages very few tetrads of spores occurred. Counts were not made but in the material examined (several thousand cells from many anthers collected at different times) probably less than 1% of pollen mother cells formed tetrads. It is not certain that a second division always takes place. In any case by the time wall formation is completed dyads are most abundant, triads are rare, and tetrads very rare.

Some of the dyad microspores grew in size, rounded off, and separated. However, sections of open flowers showed that up to that time most of the dyads had remained small and many were flattened.

Microspores

Sections of anthers of U.S.D.A. 44488 containing young microspores were examined. Many rounded microspores of varying sizes were seen (Fig. 22). Most of the dyad cells apparently had rounded off and separated. The nuclei of these spores were far from uniform in appearance. The two nuclei found in some of the young microspores (Fig. 4) were doubtless derived from the second meiotic division, i.e., a division of a spore nucleus to form a tube and generative nucleus was not responsible.

In 100 young microspores of U.S.D.A. 44488 the nuclei appeared as follows:

Nuclei	Number of spores
Single round nucleus	23
Round nucleus and one or more micronuclei	2
Single lobed nucleus	61
Lobed nucleus and one or more micronuclei	4
Two or more nuclei nearly equal in size	10
Total	100

At the young microspore stage few dyads were visible. The young microspores assumed a spherical or almost spherical form and were present in rather a diversity of sizes. Nuclei were variable in size and shape. Lobed nuclei and micronuclei were not uncommon.

Sections of anthers of open flowers showed some normal appearing large pollen grains. The average diameter of 50 such grains was calculated to be 25 μ as compared to an average diameter of 28.7 μ in one pollen-fertile line and 26.2 μ in another. The total number of spores present in the locules was less than in fertile lines judging by the numbers present in the sections. A count of 50 sound-appearing grains to 82 empty was made in one longitudinal section. Some greatly enlarged dyads were seen. The microspores were probably formed mainly from dyads. No test of germinability has been made but it is likely that it was very low.

The locules of this variety are large and roomy. Remains of the tapetum form a very thin layer along the locule edges. Of the other varieties tested only the pollen-fertile lines had large locules.

In the Early Ohio variety the locules were small and usually contained a large quantity of disorganized tapetal material along with spores, many of which (about one-half) were empty; many of these were flattened. Of the remainder, dyad groups having a diameter of 15 to 21 μ were most common but there were also sound appearing pollen grains here and there. These were mostly about 21 μ in diameter.

In the U.S.D.A. 46000 line the locules, as in Early Ohio, were small at the open flower stage and contained considerable quantities of broken down tapetum. At this stage many small dyads were found. Microspores that occurred singly were almost all small and empty. Hundreds of anthers were examined. It is probably safe to say that in them less than 1% of the grains were sound.

Sectioned anthers of pollen-fertile lines at young free microspore stages showed, in general, spores uniform in size, each containing a single nearly spherical nucleus. Spore abortion (usually less and in some lines much less than 50%) frequently occurred late, after the spores had reached normal pollen grain size. In sections of nearly mature anthers of the pollen-fertile Minn. 82–11, 55% of the pollen grains were empty. Most of the empty grains were as large, or almost as large, as the sound appearing grains. Anthers of corresponding age from the sterile forms always contained spores in a great variety of sizes, aborting spores ranging from extremely tiny to larger than "normal" size. The sections of Minn. 82–11 revealed another peculiarity,—in some parts of the locules all the spores had aborted, in other "pockets" nearly all the grains were sound. This suggests that the abortion of microspores to the extent of about one-half in this variety may be due to slight structural or physiological defects in parts of the anthers rather than to genetic factors segregating in the meiotic divisions.

Seed Production and Seedlings

All three of the male-sterile lines proved to be capable of forming seeds when sound pollen was applied to receptive stigmas. Flowers were formed on Early Ohio only in 1939. In that year fruits developed following pollination with Minn. 82–11 and U.S.D.A. 45075. Each year most of the open flowers

of U.S.D.A. 44488 were pollinated without success until 1940 when two fruits were matured following pollination with Minn. 75–5. Each fruit contained many seeds indicating that embryo sac abortion was not extensive. U.S.D.A. 46000 plants have formed a few fruits. As the number of seeds in fruits was always small, there may be some female sterility in this line. The pollen parents in successful crosses involving this line were the excellent pollen producers Minn. 11–1–2–1 and 75–5. Premature abscission of flowers is probably the chief obstacle to obtaining seeds in all three varieties.

Ten seedlings of U.S.D.A. 46000 × Minn. 11–1–2–1 were grown for two consecutive years. Tests of pollen production and soundness were made. All the plants bloomed. Only three failed to yield any pollen when the ripe anthers were tapped. Three produced abundant pollen. One plant produced as high a percentage of sound pollen as the male parent (about 90%). One produced fruits following open pollination. It is probable that at least four out of 10 F_1 plants produce sufficient viable pollen to be used successfully in back-crosses to the male-sterile parent variety. Such a procedure may be desirable when the male-sterile variety is commercially valuable but has one or a few undesirable features not found in the pollen parent of the original cross.

Discussion and Conclusions

The formation of viable functioning potato pollen may be prevented by bud abscission, meiotic failure, or abortion of spores after tetrad formation. The importance of the first of these was recognized by Stout and Clark (4) who recognized also the importance of environmental factors, especially temperature, in influencing this abscission. Numerous reports on meiotic failure have appeared (1, 2, 5, 6). These have shown that at least in some varieties univalents are present at metaphase of the first meiotic division and that chromosome distribution to the two poles is not always equal. Monads may form by complete failure of the first division to occur or by fusion of chromosome groups after partial or complete separation. Dyads may result from complete failure of the second division, from fusion of the two second division spindles, and possibly in other ways. The observations made on the first meiotic division in connection with the present work indicated that failure of the first meiotic division was not conspicuous. Most of the failure observed became evident at the end of the first division or later. There probably are cases, however, in which failure of the second division is due to irregularities in the first.

In the male-sterile varieties studied the second division is considerably delayed in comparison to varieties that normally form tetrads. Possibly the first division is also delayed (1) although anther measurements of sterile and fertile lines failed to reveal any consistent size differences up to anaphase stages. The second division is perhaps omitted entirely in some cells. In many, however, a delayed second division may be observed. In these the two nuclei are usually close together near the centre of the cell by the time the equatorial plate stages are reached. Thus the nuclei are unable to divide

independently of each other. It is suggested that the delay in the second division results in failure to carry it through successfully, that is, a disturbance of the timing mechanism is a cause of male sterility. It is perhaps as likely that the failure of the second division is caused by some disturbance of the physiology of microsporocytes as by chromosome irregularities owing to the hybrid condition of the plant.

The lines or varieties classed as male-fertile are those that form mainly microspore tetrads at the conclusion of the meiotic divisions. In all of these lines the percentage of sound pollen grains fluctuates rather widely. If spore lethals were the sole cause of spore abortion little fluctuation should occur. Factors other than spore lethals must frequently operate to increase the proportion of aborted spores in such lines.

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A STUDY OF THE RELATIVE EFFICIENCY OF SEED SAMPLING METHODS[1]

By C. W. Leggatt[2]

Abstract

Using prepared samples of seed, in which impurities were represented by stained seed of the same kind, repeated samplings from a restricted bulk were made by the method being studied. After each sample was drawn, the number of stained seeds in it was recorded and it was then put back in the bulk. The data, being in the form of numbers in a unit weight, were first compared with the corresponding Poisson distribution, which, however, was found inapplicable. Working from first principles, an expression was found that was believed to suit the conditions of the experiment and that proved to be a special case of the binomial distribution, not previously applied, it is believed, to studies of this sort.

Statistical tests showed an excellent fit between observed results and the values expected according to the new expression, which thus provides a measure of the variability to be expected in drawing subsamples from a restricted bulk and at the same time provides the required basis for comparison of the seed sampling methods under study. It shows that less variation is to be expected between duplicate tests of a submitted sample than was previously thought to be the case.

Introduction

In the control of a commodity such as seed, which is notoriously difficult to sample satisfactorily, the question of methods of sampling assumes a prime importance. This is true not only of sampling from the bulk but also of the drawing of a satisfactory working sample from a sample submitted to the laboratory for test, which this forms the subject of this paper. The procedure adopted in the study was to compare the results of a large number of experiments by any particular sampling method with what would be expected on statistical grounds.

The laboratories referred to are those of the Laboratory Service, Plant Products Division, Dominion Department of Agriculture, Canada.

Materials and Methods

Briefly, the procedure followed was to prepare samples of seed in which impurities were represented by stained seed of the same kind; from these samples, which represent restricted bulks, repeated subsamplings were made by the method being studied. During the summer of 1939, samples of the following crop plants were distributed to various laboratories for studies as indicated:

[1] *Manuscript received Feb. 8, 1941.*

Contribution No. 656, from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa. Project S.S. B. 18.02.

[2] *Botanist (Physiology).*

Samples of:	Sampling method
Wheat and oats	
Stained seeds to:	
Calgary	Boerner
Toronto	Pouring*
Seed Research Laboratory, Ottawa	Boerner
Sweet clover	
Two 40-gm. samples each containing 80 stained seeds to:	
Sackville	Wright
Montreal	Wright
Ottawa	Wright
Seed Research Laboratory, Ottawa	Leggatt (2)
Winnipeg	Wright
Saskatoon	Wright
Vancouver	Wright

** The method referred to consists of mixing the submitted sample by pouring it back and forth between two large containers. The working sample is obtained by pouring the required weight on to the pan of a scale.*

The method of obtaining experimental data was the same for both cereals and clover. In the cereals, 32 of the stained seeds supplied were to be added to 4 lb. of seed of the same kind. This constituted the "submitted" sample corresponding to the 40-gm. samples of sweet clover.

After an initial mixing the submitted samples were to be repeatedly divided down by the mixer (or method) in question; samples were taken from alternate spouts, until four cuts had been made, providing a working sample 1/16 as large as the submitted sample. This working sample was spread on the analysis table, the number of stained seeds recorded, and then it was returned to restore the original submitted sample. This process was repeated, but without the initial mixing, until 1000 trials had been made. The data have been summarized and are given in Tables I, II, and III.

Theoretical Considerations

Previously, when considering data of this type, i.e., numbers of impurities per unit weight, it has been assumed that the Poisson distribution has been the proper criterion for comparison of the observational data. It has been noted on more than one occasion, however, in similar studies (1) where observed data which followed the binomial distribution were compared with the expected values, that there is a tendency towards a narrowing of the distribution curve, i.e., more values were observed near the mean and fewer towards the extremes than were expected. This tendency was ascribed to the probable effect of the restricted size of the bulk sample. Preliminary studies of the present data strongly indicated a similar tendency, so much so that it was felt that the matter should be studied further.

It is implicit for the validity of the Poisson distribution that the sample be drawn from an indefinitely large bulk; thus there is no restriction on the possible

number of impurities that may be picked up in any sample except the value of n, i.e., the size of the sample itself. However, the Poisson theorem postulates that n is indefinitely large compared to p (the probability of the occurrence of the event, i.e., of an impurity) so that in effect no restriction exists.

For the data used in this study, however, the bulk from which the working sample was drawn is very far from being indefinitely larger than the latter. It is, in fact, only 16 times as large, the subsample being the result of four successive divisions-in-two, or dichotomies, D.

In the bulk or submitted sample from which the subsample was drawn the number of impurities is sharply limited. Since the bulk sample at the first dichotomy, D_1, is divided into two equal parts, the chance of any given seed falling into one or other of the halves is $\frac{1}{2}$, or $p = .5$. At the second, D_2, the chance of its falling into any one of the four possible subsamples is $\frac{1}{4}$ or $\frac{1}{2^2} = \frac{1}{2^d}$, where d is the order of dichotomy. Similarly, at D_3, $p' = \frac{1}{8} = \frac{1}{2^3} = \frac{1}{2^d}$; and so on for any number of dichotomies, $p' = \frac{1}{2^d}$.

Since $q' = 1 - p'$, we have $q' = 1 - \frac{1}{2^d} = \frac{2^d - 1}{2^d}$ and since $m = n'p'$ we have $m = \frac{n'}{2^d}$ and also $n' = 2^d m$ which, of course, is the number of impurities in the bulk sample.

The distribution, then, is given by the expansion of $(p' + q')^{n'}$ where the symbols have the above meanings. This is an expression of the ordinary binomial form which has a variance, $V = n'p'q' = n' \cdot \frac{1}{2^d} \cdot \frac{(2^d - 1)}{2^d}$.

Now, as the number of dichotomies becomes very great, the expression $\frac{(2^d - 1)}{2^d} \rightarrow 1$ and n' becomes indefinitely large in comparison with m, while p' becomes indefinitely small. Thus this distribution merges with the Poisson distribution when the working subsample becomes an indefinitely small fraction of the bulk sample, as would be expected.

In the following tabulation certain statistics are collected for reference and comparison. The broken infinity symbol $\propto$ is used to indicate an indefinitely large quantity, not however reaching infinity.

Distribution	Expansion of:	Variance	m	p	q	n
Binomial	$(p + q)^n$	npq	np	<1	$1 - p$	Any value
Poisson	$(p + q)^n$	np	np	$\rightarrow 0$	$\rightarrow 1$	$\propto$
Binomial derived by dichotomy	$(p' + q')^{n'}$	$\frac{n'}{2^d} \cdot \frac{(2^d - 1)}{2^d}$	$\frac{n'}{2^d}$	$\frac{1}{2^d}$	$\frac{2^d - 1}{2^d}$	$n' =$ any value

Note: d = the number of dichotomies.

Having worked out the foregoing, the results obtained in this series of experiments were compared with the expected "dichotomous" binomial distributions, using Fisher's χ^2 test of goodness of fit. The results, which were found to concord well with the new theory but not with the Poisson, are given in the next section.

The "dichotomous" binomial distribution curve proves to be narrower than the Poisson curve and explains the anomalies previously referred to. It is of practical significance in that it provides a measure of the variability to be expected in drawing subsamples from a restricted bulk and shows that less variation is to be expected between duplicate tests or analyses of a submitted sample than was previously believed to be the case. It also gives the proper criterion for making such comparisons as form the subject of this paper.

The distribution curve which is discussed above and which is obtained as the result of drawing samples from a comparatively small bulk or submitted sample is not restricted to the case where there is a succession of even dichotomies. It will be noted that the value $p' = \frac{1}{2^d}$ is simply the fraction of the submitted sample constituted by the working sample.

Let w = size of working sample
s = size of submitted sample
then

$$p' = \frac{w}{s}$$

$$q' = 1 - \frac{w}{s} = \frac{s - w}{s}$$

$$\therefore m = n'p' = \frac{n'w}{s}$$

$$V = \frac{n'w}{s} \cdot \frac{s - w}{s}$$

These symbols may be used when the working sample is any fraction, not necessarily an even submultiple, of the submitted sample.

Experimental Results

Sweet Clover

In Table I are presented the combined results obtained by the seven laboratories which analysed the sweet clover samples.

It will be seen that the "dichotomous" binomial distribution fitted the observed distribution very well but that the Poisson did not do so. This confirms the validity of the new theory as a basis for the comparison of results from restricted bulks and at the same time shows that the sampling methods used were, on the whole, satisfactory.

Of the seven stations taking part, six used the Wright sampler and one used the Leggatt sampler. In Table II the results of the χ^2 test are given to enable comparison of the two types of sampler and of the two theoretical distributions to be made.

TABLE I

RESULTS OF 7000 ANALYSES OF $\frac{1}{4}$ OZ. SAMPLES REPEATEDLY DRAWN FROM BULK SAMPLES OF 4 OZ. OF SWEET CLOVER CONTAINING 80 STAINED SEEDS, COMPARED WITH THE CORRESPONDING POISSON AND "DICHOTOMOUS" BINOMIAL DISTRIBUTIONS

Number stained seeds found	Number of analyses giving or expected to give numbers of stained seeds listed in Column 1		
	Observed	Poisson	"Dichotomous" binomial
0	46	50	43
1	212	246	225
2	560	608	585
3	1004	1002	1000
4	1265	1240	1267
5	1315	1228	1268
6	1051	1013	1043
7	725	716	726
8	447	443	436
9	220	244	229
10	86	121	107
11	37	54	45
12	18	22	17
13	12	9	6
14	2	3	2
15	–	1	1
TOTAL	7000	7000	7000
Mean	4.94	4.95*	4.94
χ^2		35.695	12.863
n		12	12
P		<.01	.39

* *Average of distributions for $m = 4.9$ and $m = 5.0$.*

TABLE II

COMPARISON OF RESULTS FROM WRIGHT AND LEGGATT SAMPLERS*

Sampler	Number tests	Compared with:	χ^2	n	P
Wright (Mean 4.94)	6000	Poisson	37.572	10	<.01
		"Dichotomous" binomial	16.257	10	.14
Leggatt (Mean 4.95)	1000	Poisson	12.317	10	.35
		"Dichotomous" binomial	11.379	10	.42

* *In order to enable direct comparisons between the values of χ^2 to be made, the tests were brought to the same number of degrees of freedom by grouping certain values. This has brought about some improvement in the values of P which, therefore, cannot directly be compared with those in Table I.*

In the following comparisons, where

P_1 = the probability that the greater value of χ^2 (χ^2_1) will be exceeded,

P_2 = the probability that the lesser value of χ^2 (χ^2_2) will be exceeded,

$1 - P_2$ = the probability that χ^2_2 will not be exceeded,

the probability that, in any two tests, as great a value as χ^2_1 will occur in conjunction with as small a value as χ^2_2 is taken as

$$4P_1(1 - P_2).$$

As between the two kinds of sampler, the values of χ^2 for the "dichotomous" binomial distribution do not differ significantly ($P = .32$ approximately). In the comparisons of the two distributions for the Wright sampler, however, the difference in the value of χ^2 is highly significant, but for the Leggatt sampler there appear to be too few tests to demonstrate any difference.

Wheat and Oats

In Table III are presented the combined results obtained by the three laboratories which analysed the wheat and oat samples. These results can be combined because the quantities involved were the same for both species. They include data obtained both by the Boerner sampler and the "pouring" method.

TABLE III

RESULTS OF 4800 ANALYSES OF ¼ LB. SAMPLES REPEATEDLY DRAWN FROM BULK SAMPLES OF 4 LB. OF WHEAT OR OATS CONTAINING 32 STAINED WHEAT OR OAT SEEDS RESPECTIVELY, COMPARED WITH CORRESPONDING POISSON AND "DICHOTOMOUS" BINOMIAL DISTRIBUTIONS

Number stained seeds found	Number of analyses giving or expected to give numbers of stained seeds listed in Column 1		
	Observed	Poisson	"Dichotomous" binomial
0	579	637	609
1	1329	1286	1298
2	1330	1299	1342
3	881	875	894
4	450	442	432
5	166	179	161
6	51	60	48
7	12	17	12
8	2	4	3
9 or more	–	1	1
Total	4800	4800	4800
Mean	2.02	2.02	2.00
χ^2		12.848	3.875
n		6	6
P		<.05	.70

It will be noted here again, that the Poisson distribution differs significantly from the observed results but that the "dichotomous" binomial gives an excellent fit.

Using the "dichotomous" binomial distribution as the basis of comparison, if the results of the "pouring" method (with 2000 values) and the Boerner method (with 2800 values) are examined separately, it is found that $P = .18$

for the former and $P = .98$ for the latter. The corresponding χ^2 values differ highly significantly from each other ($P = .01$); this indicates that the Boerner method is preferable although it cannot be considered that the pouring method is entirely unsatisfactory. The latter has given a great excess of tests showing one stained seed and a deficiency of 0's, although the rest of the values (two or more) provide an excellent fit with expectation. It is probable that the pouring method would not be reliable for very low rates of occurrence of the impurity.

Acknowledgment

The author would like to express his thanks to Dr. J. W. Hopkins, Biologist, National Research Council, for reading the manuscript and for suggested changes.

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CHANGES IN THE PHYSICAL PROPERTIES OF GLUTEN WITH AGING OF FLOUR[1]

By J. D. McCaig[2] and A. G. McCalla[2]

Abstract

The physical properties of gluten, as determined by the hydration of the gluten between pH 4 and 7, are deleteriously affected by aging of flour. Similar effects are obtained by adding linolic acid, whereas some of the effects of aging can be removed by extraction of the flour with ether. The original quality of the gluten is not restored by such extraction. Gluten from aged deteriorated flour swells enormously in 0.1 *N* acetic acid; such hydration is not an indication of good quality but rather of resistance to dispersion. Some freshly milled flours produce gluten possessing the physical characteristics of gluten from aged deteriorated flour.

It is concluded that the quality of gluten depends to a considerable extent on the nature of the adsorbed lipoids. Many of the characteristics of gluten are determined by relatively insoluble (unidentified) lipoid substances, whereas the absence of such lipoids and the formation of fatty acids during aging are deleterious to gluten quality.

Introduction

For the past seven years studies concerned with the effects of aging on the quality of wheat and flour have been carried on in this laboratory. Some of the earlier results have been published (1, 19), and have shown that the changes in quality of the flour with aging are intimately associated with changes in the physical properties of the gluten. This has, of course, been demonstrated by other workers (12, 22). Although gluten has been the subject of many investigations, the existing knowledge was inadequate to afford an interpretation of many of the results which we had obtained, so an intensive study of this substance was undertaken.

The extent of the work done with gluten is indicated in the Bibliography of Baking Quality Tests (11) published in 1934. This summary lists nearly 200 references to work in which gluten, directly or indirectly, was the subject of investigation. Most of these studies, particularly those of more recent years, were concerned with finding a simple, rapid test, to be carried out on flour or gluten, that would replace the baking test in evaluating the quality of wheat. Such a test has not been found, but our knowledge of gluten has been considerably extended. Only a few papers dealing with the properties of gluten can be considered here.

The view that gluten is made up of two proteins, glutenin and gliadin, and derives its physical properties from the properties of these two proteins (16, 23), is no longer widely held. The work of Sorensen (20) has shown that most

[1] *Manuscript received February 11, 1941.*

Contribution from the Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada. Paper No. 182 of the Associate Committee on Grain Research of the National Research Council and Dominion Department of Agriculture.

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soluble proteins are not made up of individual chemical compounds but rather of what he termed component systems, in which the various components are held together more or less firmly by secondary or residual valencies. The individuality of glutenin and of gliadin has been widely questioned (3, 14, 21), and it seems likely that Sorensen's concept may be extended to include the protein of gluten. Sorensen also discussed the importance of lipoids in determining the physical properties of the serum proteins, and it has been shown that these substances play an important part in determining the physical properties of gluten (12, 19, 22).

Thirty years ago, Wood and Hardy (23) discussed the amphoteric nature of gluten protein and the conclusions they reached are, as far as they went, acceptable today with little modification, although their conclusions regarding the factors determining the physical properties of gluten are not. More recent studies (4, 5, 8, 9, 10, 15, 18) have added considerably to the views expressed by Wood and Hardy. Gortner and his co-workers (9, 10, 18) showed that the properties of gluten were not determined by the acid–salt balance in the flour or the gluten, but that there were inherent differences in the properties of gluten from different wheats regardless of the amounts or kinds of acids and salts present. These conclusions were reached as a result of studies involving the swelling of gluten in acid–salt solutions (9) and of viscosity studies (10, 18). The latter were considered the more satisfactory. It was concluded that weak gluten had a lower rate of hydration than strong gluten and that it changed from a gel to a sol at a lower degree of hydration, that is, it had a lower maximum hydration capacity. Newton and Cook (15) studied the bound-water of flour suspensions and concluded that hydration rates and capacities offered an inadequate explanation of the differences in properties of strong and weak gluten. They suggest that structure as well as hydration of gluten must be considered. Larmour and Sallans (13) obtained high correlation coefficients between viscosity of flour suspensions and loaf volume (bromate formula) with a series of Marquis wheat samples varying in protein content from 8.2 to 18.3%. Had these investigators been working with samples of several varieties and a narrower range of protein content, it seems certain that the significance of the correlations between viscosity and loaf volume would have been much lower.

The same principles that underlie the methods already mentioned (9, 10, 18, 23) also underlie the patented processes of Berliner and Koopman (2) and Ruemele (17). The former process utilizes the variability of the swelling of glutens in weak acid to classify flours as weak or strong, whereas the latter uses variability in the viscosity of gluten dispersions of two concentrations in $N/50$ lactic acid. A full discussion of these methods cannot be undertaken here but other workers have not been able to substantiate all the claims made by the originators (8), although there are certainly measurable differences in different glutens.

The work most pertinent to the present study is that of Bungenberg de Jong (4, 5), on the behaviour of gliadin–glutenin mixtures at various levels

of hydrogen ion concentration. While it cannot be considered that his gliadin and glutenin were definite individual compounds, the importance of the work is hardly affected by this fact. He found (5) that the properties of mixtures were not the sum of the properties of the two components when the protein sols (in 0.001 *N* sodium hydroxide plus phosphate buffers) were between the pH values of 5.3 and 6.6, which he determined as the isoelectric points of his protein preparations. Between these two pH values one preparation was electronegative and one electropositive; mixing the two tended to neutralize the charges. The mixtures showed the greatest turbidity at different pH levels depending on the proportion of the two preparations used. It was concluded that there was an interaction between the two preparations in the pH range between the isoelectric points and that complete separation of the components could not be expected within this range.

If gluten as a whole is considered as a component system (20), then Bungenberg de Jong's preparations must be regarded as two main groups of components and the isoelectric point of each as the point at which the negative charges of some of the components just balance the positive charges of the remainder. If his two preparations were recombined in the proportions originally occurring in gluten, the isoelectric point (if it can be termed that) would be the point of balance between total positive and total negative charges. This point Bungenberg de Jong believes to be at pH 6.0 or 6.1.

In a previous paper (19) it was reported that the water absorbing capacity of glutens from deteriorated flour was lower than that of glutens from flour of good quality, between the pH values of 4 and 7.5. There also appeared to be a difference in behaviour of the two types of gluten as the pH changed from 6.8 to 7.5. At the time no explanation for this difference was offered but the studies have been continued and form the starting point for the work reported in this paper.

Material

The flour quality data that are pertinent to the present study are given in Table I. Gluten was washed by the method of Dill and Alsberg (6); acidity was determined by the Greek method (7), using tincture of curcuma as indicator. Since a discussion of particular comparisons is made throughout the paper, these data are not elaborated here. All flours except No. 11 were experimentally milled long patents, and were unbleached. The flours which were stored in the laboratory were kept in sealed containers at a moisture content of approximately 10 to 12%. Since this was not a study of storage effects, details of storage conditions are omitted.

A supply of the wheat from which No. 6 was milled in the spring of 1937 was stored in a cool bin and samples remilled in the spring of 1938 and 1939. Flour from the first milling was stored in a sealed container for two years and then restudied. Another lot of the same flour was stored for one month at 40° C. over saturated ammonium sulphate in a desiccator. This treatment raised the moisture content to approximately 20% and, to prevent the action

of moulds and bacteria, the interior of the desiccator was rinsed with toluene. A small container of toluene was also kept inside. At the end of the month this flour showed all the characteristics of an aged, deteriorated flour and was studied in comparison with the original.

TABLE I

DATA PERTAINING TO FLOURS USED IN STUDY

Flour No.	Variety	Time from milling, months	Protein in flour, %	Acidity, %*	Wet gluten, %**	Quality of wet gluten	Dry gluten, %**	Loaf volume cc.***
Main series								
1	Garnet	30	9.7	0.83	33.6	Very poor	11.7	340
2	Garnet	6	8.9	0.83	20.5	Very poor	8.0	480
3	Mixed	54	13.8	0.75	41.2	Very poor	16.0	430
4	Garnet	18	10.9	0.64	30.8	Poor	11.8	435
5	Garnet	6	13.5	0.55	41.6	Fairly good	14.6	668
6	Reward	2	15.0	0.27	55.0	Excellent	18.4	985
11	Soft wheat†	—	6.9	0.29	18.9	Soft	6.4	410
Special series								
7	Garnet	6	8.9	0.65	25.2	Poor	9.6	439
8	Garnet	6	13.4	0.49	40.7	Fairly good	14.8	657
9	Red Bobs	6	8.5	0.31	25.2	Fairly good	9.2	564
10	Red Bobs	6	14.3	0.43	47.0	Good	16.2	874

* *Expressed as percentage C_{18} fatty acids.*
** *Obtained at pH 6.8.*
*** *Malt-phosphate-bromate formula.*
† *A commercial cake flour.*

Results

Preliminary Experiments

The preliminary experiments involved a study of the effect of the pH of the washing solution on the water content of gluten and of the swelling in 0.1 *N* acetic acid of gluten from a wide variety of flours. Gluten balls were washed from sufficient flour to yield approximately 2.5 gm. of wet gluten. The washing solutions contained 0.1% phosphate buffer varying from pH 3.7 to 8.5. The gluten was dried in a vacuum oven at 98° C. for 24 hr. The experiments on the swelling of gluten in 0.1 *N* acetic acid were based on the method of Gortner and Doherty (9). Individual gluten balls washed at pH 6.8 instead of cut discs were used. Preliminary tests showed that gluten from deteriorated flour had enormous hydration capacity in acid and the use of more dilute or weaker acids resulted in prolonged swelling times. For this reason 0.1 *N* acetic acid was used.

The results of the experiments in which gluten was washed out using solutions of pH 3.7 to 8.5 are expressed in terms of water content of the wet gluten and are presented in Fig. 1. These results are comparable to those presented by Sinclair and McCalla (19) but are much more comprehensive.

Two main conclusions emerge from this experiment. Both the hydration capacity of glutens from the different flours and the pH at which the gluten exhibits a minimum hydration, decrease with the quality of the gluten (see Table I for gluten quality notes). The gluten from soft wheat flour (No. 11) had the highest water-holding capacity of any of the glutens, and was very soft and extensible, but lacked elasticity. The gluten from flour No. 6, typical of that obtained from high grade, strong, hard, red spring wheat, possessed excellent elasticity, and was also firm and extensible regardless of the pH of the washing solution. The glutens from Flours 1, 2, and 3 were coarse, open, and very short. Those from Nos. 4 and 5 were intermediate in quality characteristics, No. 5 being decidedly better than No. 4. The "weakness" of flour No. 11 was very different from the "weakness" of Flours 1, 2, and 3, since the physical nature of the gluten was entirely different.

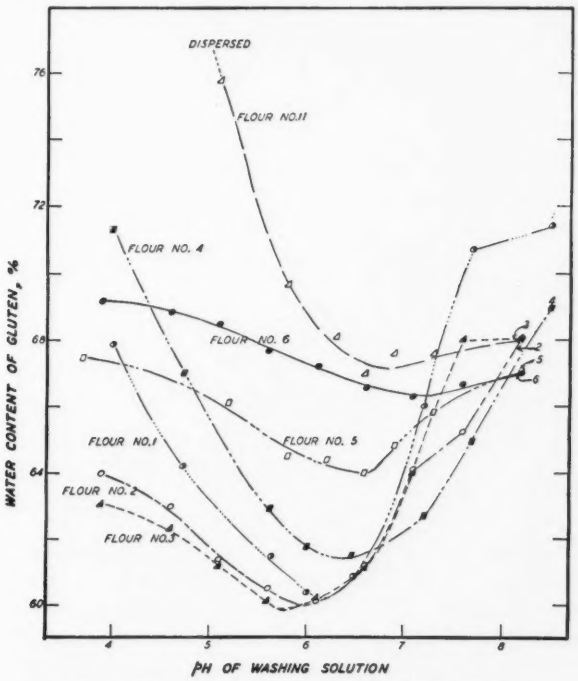

FIG. 1. *The effect of pH on the hydration of gluten.*

The results of the gluten swelling experiments expressed as increases in percentage water in the gluten are presented in Fig. 2. These results present a completely different picture, since the poor quality glutens have much the higher hydration capacity in 0.1 *N* acetic acid. These poor quality glutens did not disperse in 2.5 hr., whereas the gluten from flour No. 6 dispersed in

less than two hr. At 2.5 hr., the swollen gluten from flour No. 2 contained only 9% dry matter, but still formed a coherent mass which could be handled. In contrast to this, the gluten from flour No. 6 dispersed while still containing almost 20% dry matter. The relation between Flours 6 and 11 is in agreement with the results obtained by Gortner and Doherty (9).

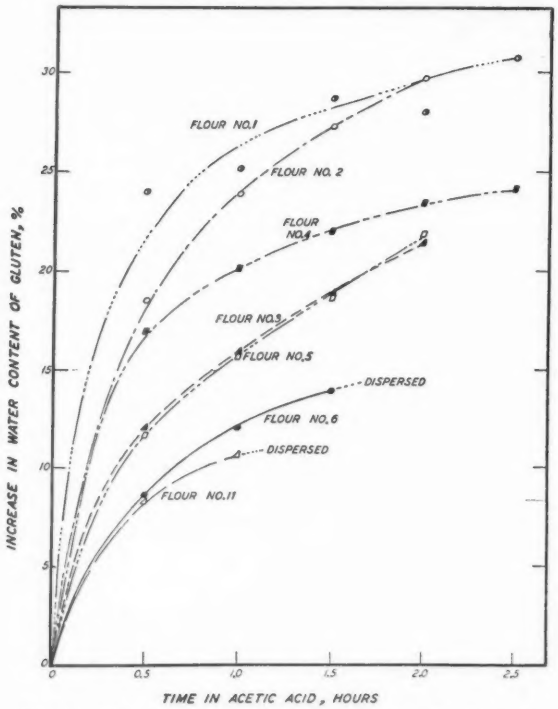

FIG. 2. *The hydration capacity of gluten in 0.1 N acetic acid.*

The enormous hydration capacity of the poor quality gluten has not, as far as we know, been reported before. It has been noted that the gluten from deteriorated flour disperses less readily in sodium salicylate than does that from high quality flour (19). The present results show that this is true in acid solutions also, and that a poor quality, freshly milled flour yields gluten similar to that of deteriorated flour, although the hydration capacity seems to be even greater at this low pH (approximately 3.0). The results obtained with the gluten from flour No. 3 are of particular interest, since this sample was just as poor in quality and hydration at higher pH values as No. 1 or No. 2, but did not exhibit the very excessive swelling of these two in acetic acid. This suggests not only that the original source and variety of the material (No. 3 was from originally high quality wheat) may affect

the swelling, but also that the factors determining the rate and extent of swelling in acid are different from those determining the hydration at higher pH values.

Effects of Acid and Salt Concentration on Gluten Swelling

That differences in the concentration of acids and salts present in gluten do not determine its physical properties has been established (9). Fisher and Halton (8), however, found that if 2% sodium chloride solution were used to wash out the gluten, differences in swelling by the Berliner method (2) disappeared. In order that our results should be comparable to those obtained by others, it was necessary to show that the differences obtained with glutens using 0.1 *N* acid would be obtained with more dilute acid and with varying concentrations of salt. Experiments were therefore carried out with Flours 1 and 6, which represented the two extremes of gluten quality. The results are presented in Table II. The glutens were washed with salt solutions of the concentration present in the acid used for swelling tests.

TABLE II

EFFECT OF ACID AND SALT CONCENTRATION ON HYDRATION OF GLUTEN, 1 HR. IMMERSION

Effect of acid			Effect of salt		
Acid concentration, normality	Increase in water, %		Salt concentration, %	Increase in water, %	
	No. 1	No. 6		No. 1	No. 6
0.001	3.0	3.8	0	25.1	12.5
0.01	12.1	10.1	0.05	22.3	—
0.02	16.5	10.9	0.1	15.2	5.0
0.05	19.5	12.3	0.2	11.9	2.0
0.1	21.8	12.5	0.5	3.0	0.7
0.5	24.8	12.3	1.0	1.5	0.4

Note: Ash in flour, %: No. 1, 0·67; No. 6, 0·40.
Ash in dry gluten, %: No. 1, 0·84; No. 6, 0·58.

The greatest differentiation between the two glutens was obtained at the highest acid concentration but the maximum swelling of the better gluten was in 0.1 *N* acid. At all concentrations except 0.001 *N* the greater hydration capacity of No. 1 was evident. Very low concentrations of sodium chloride reduced the swelling of the glutens, but their relative positions were maintained. The differences in the original properties of the gluten could not be due to the salts present, since the gluten that swelled the more was decidedly higher in ash.

It is concluded, therefore, that the differences noted among the glutens (Fig. 2) would have been obtained had the methods of Gortner and Doherty (9) been followed exactly.

Berliner and Koopman Method

The method of Berliner and Koopman (2) involves the same principles as does that of Gortner and Doherty (9), except for the method of measurement. Berliner and Koopman consider that a high swelling number indicates a good quality gluten. Gluten balls from Flours 1 and 6 were tested using this method, as outlined by Fisher and Halton (8). One-gram pieces of wet gluten were used and swelling was permitted to proceed for 5 hr. The results are presented in Table III.

TABLE III

SWELLING NUMBERS OF GLUTEN (BERLINER AND KOOPMAN)

Time, hr.	No. 1	No. 6	Time, hr.	No. 1	No. 6
0			2.0	27.1	7.8
0.5	11.8	4.1	2.5	30.0*	9.8
1.0	17.6	5.1	3.0	—	11.1
1.5	23.7	7.7	5.0	—	16.9

* *Maximum possible to measure.*

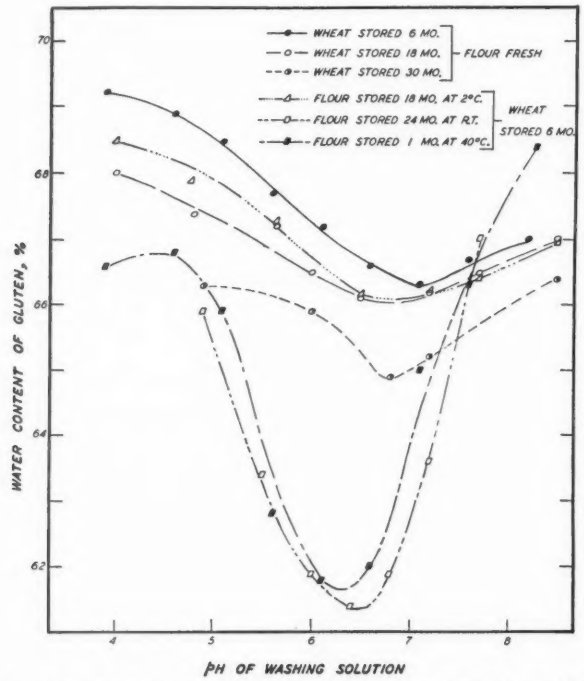

FIG. 3. *The effect of pH on the hydration of gluten from stored flour and wheat.*

By the end of 2 hr. the gluten from both flours had disintegrated to a considerable extent but measurements were continued. It is obvious that the swelling number does not represent the real volume of the swollen gluten as a large amount of water is entrapped among the pieces of gluten. The difference in behaviour of the two glutens agrees with the results obtained with the other method, and according to Berliner and Koopman shows that the gluten from flour No. 1 is decidedly the better of the two. This is, of course, incorrect. At the end of 22 hr. the gluten from flour No. 6 was dispersed, but that from flour No. 1 was granular. The particles were fine and did not settle readily. This behaviour is of the same type as is obtained when dispersion of similar glutens in sodium salicylate is attempted.

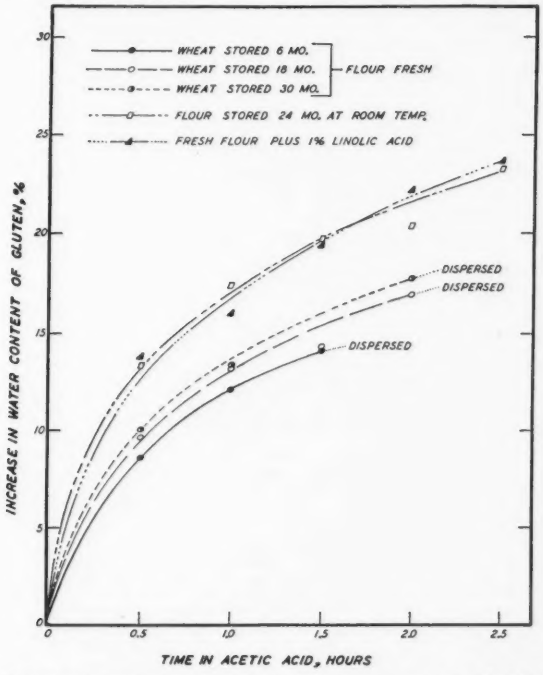

FIG. 4. *The hydration capacity of gluten in 0.1 N acetic acid as affected by storage of wheat and flour.*

Effect of Aging of an Individual Flour on Water Absorption of Gluten

The results in Figs. 1 and 2 showed a marked difference in the physical properties of gluten from fresh and deteriorated flours. Each of these flours was of different origin, however, and equally large differences were obtained with relatively fresh flours (compare Nos. 2 and 6). Flour No. 6 was therefore studied over a period of 24 months, to determine the changes that take place

in aging. A portion of this flour was artificially aged (see Material) and used in a similar study.

The results of the gluten washing tests are given in Fig. 3 and of the gluten swelling tests in Fig. 4. There was insufficient of the artificially aged flour for the latter test.

Two years' storage of the flour resulted in pronounced changes in the physical characteristics of the gluten; these changes resulted in a gluten very similar to those from any of the aged flours illustrated in Figs. 1 and 2. Artificial aging for one month at 20% moisture and 40° C. had the same effect. As with the poor quality glutens of Flours 1, 2, and 3, both the hydration capacity in buffer solutions and the pH at minimum absorption were reduced with aging. The glutens were coarse and open, lacked extensibility, and were very short. The loaf volume of the bread baked from this flour (naturally aged) had decreased by nearly 30% from that of the original.

The results also show that storage in the form of wheat resulted in gradual changes in the properties of the gluten but that these changes were much less than when Flour was stored. A small amount of the flour from this

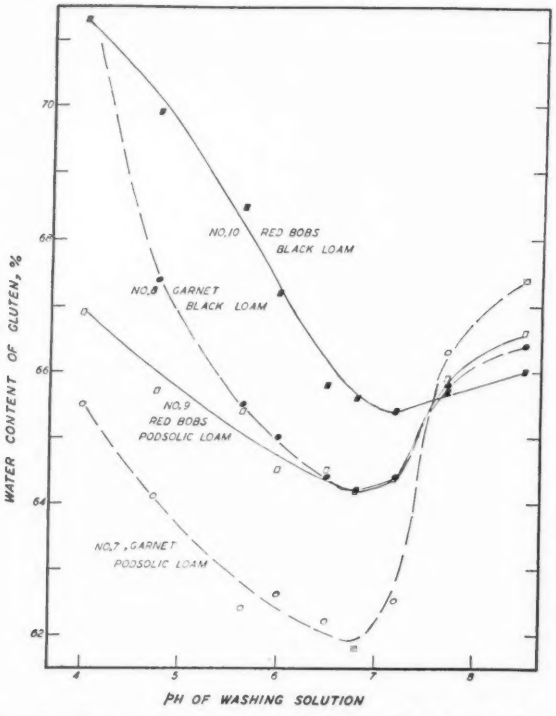

FIG. 5. *The effect of origin and variety of wheat on the hydration of gluten.*

wheat had been stored for 18 months in a refrigerator at 2° C. and was used in gluten washing studies. The results (Fig. 3) show that the change in gluten properties was small as compared with storage of the flour at room temperature.

Effect of Variety and Origin of Wheat on Gluten

The results in Figs. 1 and 2 showed also that different samples of freshly milled flour yielded glutens of widely different qualities. Much of the work done in this laboratory recently has been concerned with differences in the quality of wheat produced on two types of soil common in Alberta—the fertile black loam typical of the Edmonton district, and the relatively infertile podsolic loam, typical of much of the northern and western parts of the province. In general the quality of wheat from the former is good, and of that from the latter, poor. This is, to a considerable extent, due to differences in protein quantity, but it has been evident that there are also differences in protein quality. These differences are illustrated in Fig. 5. Flours 7 and 9 are from Garnet and Red Bobs wheat, respectively, grown on the podsol, whereas Flours 8 and 10 are from the same varieties grown on the black loam.

Not only do these results illustrate the differences in the gluten from wheat grown on the two soils, but also the differences in the glutens from the two varieties. These differences are quite in accord with baking results, except that the protein quantity factor has here been removed.

Effects of Ether Extraction and Linolic Acid on Gluten

The results presented in Fig. 4 suggested that the changes in physical properties of gluten with flour deterioration are paralleled by the changes resulting from the addition of linolic acid. The effect of fatty acids on gluten quality has been discussed in an earlier paper (19). It was concluded that the accumulation of these acids was only one factor in determining the quality of aged flour, and that the breakdown of a complex between the protein and the more insoluble lipoids was more important, at least as far as baking quality was concerned.

Samples of Flours 3 and 6 were ether extracted (19) and the gluten then washed, using solutions of various pH values. Linolic acid was added to other samples of ether extracted flour in such quantity as to compensate for the fatty acids removed by ether extraction. The results of these tests are given in Fig. 6.

Ether extraction increased the hydration of gluten from flour No. 6 at all pH values, and of the gluten from flour No. 3 at all values below pH 6.5. This improvement in hydration is attributed to the removal of fatty acids by the extraction. The gluten from extracted flour No. 3 was still coarse, open, and short, indicating that the extraction had not removed all the effects of aging. Such extraction, therefore, did not bring the gluten of flour No. 3 back to its original properties, as this flour was originally of excellent quality, quite comparable to flour No. 6. The addition of linolic acid to compensate

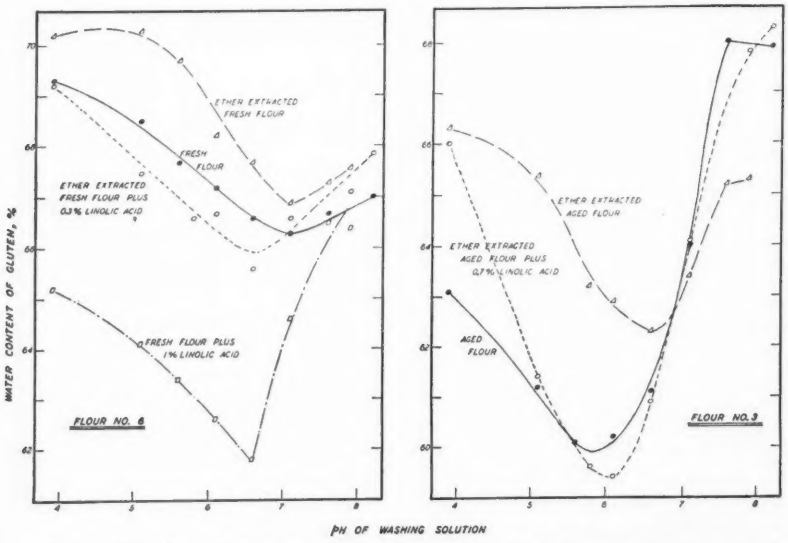

FIG. 6. *The effects of ether extraction of flour and addition of linolic acid on the hydration of gluten from fresh and aged flour.*

for the extracted acids made the gluten from extracted flour comparable to that of the gluten from the unextracted.

These results substantiate the earlier hypothesis (19), that there are two factors determining the quality of the gluten from deteriorated flour, and that the more insoluble lipoidal substances present in the flour compete with fatty acids in determining the type of gluten complex formed on washing. If the fatty acids are extracted, gluten quality is improved, but unless the important lipoids are available, good quality gluten cannot be obtained.

Discussion

The conception of gluten as a complex of protein and lipoids has been discussed in an earlier paper (19) and by others (3). It was suggested that the physical changes in gluten that occur as flour ages are due to two factors: first, the breakdown of the complex and second, the accumulation of end products, particularly the unsaturated fatty acids. As a result of further studies this hypothesis needs some modification. Gluten is a protein-lipoid complex but this complex is apparently formed when a dough is made or the gluten is washed from the flour. It is, therefore, probably incorrect to speak of the breakdown of this complex with aging of the flour, since it is more likely that a change in the lipoidal substances themselves renders them incapable of conferring upon the gluten mass the properties of coherence and extensibility. As flour ages the breakdown of lipoidal compounds also results in the accumulation of fatty acids, which compete for the adsorption bonds in the formation of the gluten.

If a particular type of lipoidal substance is necessary to produce high quality gluten, the results obtained in this study may be interpreted in the following way. High quality flour produces coherent, extensible, and elastic gluten because the essential lipoids are adsorbed on the protein of the gluten. When fatty acids are added to a high quality flour, they compete with the lipoids for the adsorption bonds and, if present in sufficient quantity, replace the other lipoids to such an extent that the gluten loses much of its coherence and extensibility. As a flour ages, the essential lipoids break down and fatty acids accumulate. Eventually the flour yields gluten which is coarse, open, and short. When the fatty acids are extracted, the quality of the gluten is improved because the acids no longer compete with the remaining lipoids, but the original quality is not regained because the breakdown of the original lipoids has proceeded too far.

In this study, another type of flour was encountered. From this (flour No. 2) good quality gluten was not obtainable even at the time of milling. It seems probable that the lipoidal substances necessary to produce a high quality gluten complex were not available in this flour; that is, they had not been metabolized.

These conclusions permit the acceptance of the hypothesis suggested by Bungenberg de Jong (5). If gluten is considered as a single colloidal complex made up of various protein components plus one or more lipoidal substances, this general hypothesis fits all the results obtained in the present study. A complex made up only of protein would have an isoelectric point at the point of balance between positive and negative charges on the components. The presence of lipoidal substances alters this point, its exact position being determined by the amounts and proportions of the various lipoids. Our results indicate that in a high quality gluten this point, which probably should not be called an isoelectric point except that the term serves to indicate a point of minimum swelling, is at or near pH 7.0. Any alteration in the balance of the lipoids taking place as the flour ages results in this point shifting to a lower pH. From Bungenberg de Jong's results (5), it seems probable that the protein complex alone would have an isoelectric point at approximately pH 6.0, and this point is about the lowest obtained in the present study. This agreement may be fortuitous, however, since fatty acids at least, and probably some unaltered essential lipoids, were present in all of the flours used in the present study.

The results of the gluten swelling experiments carried out on these flours are determined, it is believed, by different factors, since pH 3.0 is well on the acid side of the isoelectric point of any of the components. The results of this test give an indication of the dispersibility of the gluten rather than any other quality factor, the most easily dispersed gluten exhibiting the slowest rate of swelling and the lowest hydration at which coherence is maintained.

Only a few remarks have been made regarding the gluten from soft wheat flour. There is no doubt that this is different from hard wheat gluten but too little has been done to characterize it accurately. The comparison of

gluten from different types of wheat is to be the object of another study. The quality of individual flours used in this study varied enormously. The study of gluten reveals great differences, but whether or not such gluten studies can be used in differentiating flours of similar type and general quality remains to be determined.

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EFFECTS OF TALC DUSTS CONTAINING PHYTOHORMONE, NUTRIENT SALTS, AND AN ORGANIC MERCURIAL DISINFECTANT ON THE ROOTING OF HERBACEOUS CUTTINGS[1]

By N. H. Grace[2]

Abstract

Cuttings of *Coleus Blumei*, varieties of *Chrysanthemum* and species and varieties of *Iresine* were treated with a series of talc dusts containing naphthylbutyric acid, nutrient salts, and ethyl mercuric bromide and then were propagated in sand in the greenhouse. Naphthylbutyric acid treatment increased the number of roots per rooted cutting, and its combination with the mixture of nutrient salts increased fresh root weight of *Coleus* cuttings. Organic mercury treatment increased, by about 5%, the number of *Chrysanthemum* cuttings that rooted, and increased the number of roots on *Iresine* cuttings. Beneficial effects from talc treatment alone were a feature of the results. Differential reactions to both talc and organic mercury treatments were shown by closely related varieties.

Earlier communications have reported on the effects of treatment of plant stem cuttings with talc dusts containing phytohormone, nutrient salts, and disinfectant chemicals (2, 3, 6, 7, 9, 10). This communication describes the results of such treatment on cuttings of three genera of herbaceous plants.

Experimental

The factorial series of talc dusts used in these experiments has been described in detail in a recent article dealing with the responses of *Taxus* cuttings (9). The dusts contained a mixture of nutrient salts at concentrations of 0, 0.1, 1, and 10%, each taken separately in talc, and in combination with 0 and 50 p.p.m. ethyl mercuric bromide and 0, 250, and 1000 p.p.m. of naphthylbutyric acid. Cuttings* of three horticultural varieties (Margaret Waite, Lillian Godfrey, and Bronze Godfrey) of *Chrysanthemum indicum* L. were treated with the entire series of 32 dusts. *Iresine* (*I. Lindeni* Vanhoutte, *I. Herbstii* Hook. f., and *I. Herbstii* var. *aureo reticulata*) received all treatments except 250 p.p.m. naphthylbutyric acid. Cuttings of *Coleus Blumei* Benth. were treated with only 16 of the dusts. Treatments with 250 and 500 p.p.m. were omitted.

Cuttings were sprinkled with water and groups of seven of *Chrysanthemum* and *Iresine* and five of *Coleus* were dipped in dust to a depth of about one-half inch. Excess dust was shaken off and the cuttings were planted immediately in a relatively coarse brown sand (8); the frames were covered with factory cotton screens for the first week after planting. The experiments were made

[1] *Communication received March 3, 1941.*

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. N.R.C. No. 990.

[2] *Biochemist.*

**Prepared cuttings of Coleus and Iresine were supplied by the Federal District Commission, Ottawa, through the kindness of Mr. E. I. Wood. Cuttings of Chrysanthemum were purchased from a local florist.*

during the months January to March, 1940. During this period the temperature in the greenhouse ranged around 65° F.

All experiments were arranged according to the principles of experimental design, with treatments replicated and groups of cuttings arranged in random order in the medium. The random arrangement permitted comparison of three varieties of *Chrysanthemum* and two species and a variety of *Iresine* and the interactions between species and varieties and the various chemical treatments. Experiments included groups of untreated cuttings to permit consideration of the effects of talc treatment. The experiments required 1512 *Chrysanthemum* cuttings, 255 cuttings of *Coleus*, and 1638 of *Iresine*. All results were subjected to the analysis of variance procedure except where the data were too meagre to warrant such treatment. Data for counts of numbers of cuttings were subjected to the inverse sine transformation prior to statistical treatment (1).

Cuttings were removed approximately four weeks after planting and record was made of the number rooted, dead, and the number of roots. The length of root mass and fresh weight of plants and of roots were determined for *Coleus* and *Iresine*. Also, total root length of *Chrysanthemum* cuttings was determined and the mean root length calculated. The number of roots and the fresh plant and root weights were calculated on a "per rooted cutting" basis.

Results

The results of the analyses of variance of data are not given. The statistical treatment was closely similar to that described in detail for a previous experiment involving use of the same chemical treatments (9).

TABLE I

AVERAGE EFFECTS OF ETHYL MERCURIC BROMIDE ON THE PERCENTAGE ROOTING OF *Chrysanthemum* CUTTINGS

Concentration of ethyl mercuric bromide, p.p.m.	*Chrysanthemum* varieties			Mean of treatments
	Bronze Godfrey	Lillian Godfrey	Margaret Waite	
0	70.5	41.1	11.6	41.1
50	75.0	44.7	17.4	45.7

Chrysanthemum

Effects of ethyl mercuric bromide on rooting are described in Table I in which the data are averages for all phytohormone and nutrient salt treatments. Although the average beneficial effect was only 4.6%, it was apparent in all three varieties. Marked differences in extent of rooting of these horticultural varieties also was evident.

Data comparing the responses of untreated and talc treated cuttings are given in Table II. Talc appeared to increase the rooting of two, and decrease rooting of one, of the varieties. Beneficial effects also were suggested by counts of numbers and lengths of root per rooted cutting.

TABLE II

EFFECT OF TALC TREATMENT ON RESPONSES OF *Chrysanthemum* CUTTINGS

Variety	Cuttings rooted, %		Number of roots per rooted cutting		Length of roots per rooted cutting		Mean root length, mm.	
	Untreated	Talc treated	Untreated	Talc treated	Untreated	Talc treated	Untreated	Talc treated
Bronze Godfrey	67.9	71.4	7.2	9.2	106	138	14.7	15.1
Lillian Godfrey	57.2	32.1	6.8	8.8	105	138	15.5	15.7
Margaret Waite	3.6	10.7						

Results of naphthylbutyric acid treatments on the number of roots per rooted cutting are given in Table III, in which the data are averages for all nutrient salt concentrations and treatments with and without organic mercury. The number of roots increased progressively with increase in concentration of naphthylbutyric acid; the increase was significant at the 1000 p.p.m. concentration. However, this significant increase in number of roots occurred in the absence of organic mercury, or when the 1000 p.p.m. concentration was in combination with both organic mercury and nutrient salts, particularly the 10% concentration of the latter.

TABLE III

AVERAGE EFFECTS OF NAPHTHYLBUTYRIC ACID ON THE NUMBER OF ROOTS PER ROOTED *Chrysanthemum* CUTTING

Naphthylbutyric acid in talc, p.p.m.				Necessary difference, 5% level
0	250	500	1000	
8.0	8.6	9.4	10.1	1.56

Coleus

Rooting of the talc treated cuttings attained 98%, that of the untreated, 93%. Naphthylbutyric acid treatment increased the number of roots per rooted cutting by 2.7 on the average, i.e., from 10.9 to 13.8. Interaction effects of phytohormone and nutrient salt treatments on fresh root weight per rooted cutting are described in Table IV in which the data are averages for treatments with and without organic mercury. The substantial increase

after phytohormone treatment is wholly attributable to the interaction between naphthylbutyric acid and nutrient treatments, particularly the 10% concentration.

TABLE IV

AVERAGE EFFECTS OF NAPHTHYLBUTYRIC ACID AND NUTRIENT SALTS ON THE FRESH ROOT WEIGHT PER ROOTED *Coleus* CUTTING, CG.

Naphthylbutyric acid in talc, p.p.m.	Nutrient salts in talc, %				Mean of naphthylbutyric treatments
	0	0.1	1	10	
0	10.9	9.8	8.6	9.4	9.7
1000	10.9	10.5	11.9	16.4	12.4

Necessary difference 5% level, for interaction: 3.8.

Iresine

The data in Table V indicate that talc treatment effected marked increase in rooting, number of roots, and fresh plant weight, and reduced mortality.

TABLE V

AVERAGE EFFECTS OF TALC TREATMENT ON RESPONSES OF *Iresine* CUTTINGS

Responses	Untreated cuttings	Talc treated cuttings
Number of cuttings rooted, %	40.5	95.6
Number of cuttings dead, %	58.0	4.1
Number of roots per rooted cutting	12.2	17.0
Fresh plant weight per rooted cutting, dg.	6.2	8.0

TABLE VI

INTERACTION EFFECTS OF TALC TREATMENT AND SPECIES AND VARIETIES OF *Iresine* ON THE FRESH PLANT WEIGHT PER ROOTED CUTTING, DG.

—	*I. Lindeni*	*I. Herbstii*	*I. Herbstii* var. *aureo reticulata*
Untreated cuttings	5.9	7.1	5.8
Talc treated cuttings	6.7	8.6	8.9

Necessary difference, 5% level: 1.3.

Differential effects of talc treatment on fresh plant weight of species and varieties are given in Table VI, significant increase in plant weight from talc treatment being shown by *I. Herbstii* and its variety *aureo reticulata* but not by *I. Lindeni*.

Naphthylbutyric acid treatment, on the average, increased the number of roots per rooted cutting from 15.2 for controls to 17.4 and 18.4 after treatment with the 500 and 1000 p.p.m. concentrations respectively, the necessary difference being 0.89 for the 5% level of significance.

TABLE VII

AVERAGE EFFECTS OF ORGANIC MERCURY ON THE NUMBER OF ROOTS PER ROOTED *Iresine* CUTTING

Concentration of ethyl mercuric bromide in talc, p.p.m.	*I. Lindeni*	*I. Herbstii*	*I. Herbstii* var. *aureo reticulata*	Mean of organic mercury treatments
0	14.2	16.9	17.4	16.2
50	16.7	19.2	17.4	17.8

Necessary difference, 5% level: 1.78.

The effects of organic mercury treatment are given in Table VII in which data for the number of roots per rooted cutting are averages over all phytohormone and nutrient salt concentrations. A highly significant increase of 1.6 in the number of roots resulted from organic mercury treatment in *I. Lindeni* and *I. Herbstii*. Treatment failed to affect the number of roots per rooted cutting of *I. Herbstii* var. *aureo reticulata*. Data for the fresh root weight per rooted cutting indicated that the variety had a substantially greater root weight than the *I. Lindeni* and *I. Herbstii* which did not differ in this respect.

Discussion

Naphthylbutyric acid treatment effected a general increase in the number of roots per rooted cutting. Combination of this growth stimulating chemical with a mixture of nutrient salts substantially increased fresh root weight of *Coleus* cuttings. Combination of the 1000 p.p.m. concentration of this chemical and 10% of nutrient salts in talc has already been shown to affect the length of new growth of *Taxus* cuttings (9). Effects of nutrient salt treatments of these herbaceous cuttings were neither as numerous nor as pronounced as with cuttings of some other species (2, 6, 9, 10).

Organic mercury treatment increased rooting of *Chrysanthemum* cuttings, though the extent of the effects was small. Organic mercurial treatments have been shown to increase the rooting of other plants (2, 10). Effects of 50 p.p.m. ethyl mercuric bromide on number of roots per rooted cutting in *Iresine* are similar to those of an earlier experiment in which the same concentration of the phosphate was used (2). It may be concluded that organic mercury treatment favourably affects responses of cuttings of only certain plants, and that there may be differences between varieties and closely related species. Although the extent of the effects is usually small, it may be

recalled that rooting of *Physocarpus* cuttings was increased as much as 19% by organic mercurial treatment (2).

The beneficial effects of talc treatment stand out as a feature of the results. Similar effects on responses of cuttings of other plant species have been reported (4, 5, 7, 11, 12). Although each of the three genera considered demonstrated increased rooting from talc treatment, one variety of *Chrysanthemum* and one of *Iresine* failed to respond favourably. Differential effects from talc treatment have been noted with spruce cuttings (7). Beneficial effects from talc treatment have been attributed, in part, to water relationships (4, 11). In these experiments relative humidity tended to be low in the greenhouse and there were some drafts of warm air owing to unit heaters. Such conditions might be particularly favourable to talc treatment.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. D. MAY, 1941 NUMBER 5

RED SORE DISEASE OF PIKE[1]

BY G. B. REED[2] AND G. C. TONER[3]

Abstract

"Red sore" disease of pike, common in these fish in eastern Ontario waters, is caused by an infection with *Proteus hydrophilus*, the organism previously shown to be responsible for the widespread "red leg" disease of frogs and probably responsible for "ulcer disease" in trout in a New York hatchery.

A disease of fish, characterized externally by red areas to open necrotic lesions of the skin on any part of the body or fins, has been known for many years in eastern Ontario. The disease, generally described locally as "red sore", has been noted most frequently by both anglers and commercial fishermen in the pike, *Esox lucius*, but other species are said to show it to a less conspicuous degree. It has been described as occurring in fish of the St. Lawrence, Rideau, and Napanee river systems in eastern Ontario. Pike from some areas in these systems appear to be free of the disease, in other regions it is apparent on most of the pike taken.

There does not appear to be any published reference to the disease. Anglers, commercial fishermen, and fish experts have expressed the opinion that it does not occur in western Ontario or in Quebec. This may indicate that the disease has only a regional distribution or it may mean that in other regions it is of relatively rare occurrence.

The disease in pike is extremely varied. In its mildest form, it varies from reddened points in the skin suggesting slight petechial haemorrhage to deep red areas several square centimeters in extent, the scales frequently being displaced. In the more severe form it appears as red, slimy, necrotic areas extending through the skin and in some instances deep into the muscle. The visceral organs, in gross appearance, are unchanged or in some instances the kidney appears darker in colour and softer in texture than the normal.

Ten pike showing the more severe superficial lesions, taken from different lakes on the St. Lawrence, Rideau, and Napanee river systems were subjected to a rather detailed bacteriological examination in an attempt to determine whether or not the disease was of bacterial origin and, if so, to isolate the causal agent. The method adopted was to make a series of cultures from each fish as soon as possible after it was taken from the water. The time

[1] *Manuscript received January 16, 1941.*
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varied from 1 to 24 hr. After more extensive procedures with the first few fish, the method adopted was to scrape the superficial lesions and introduce the scrapings into meat infusion broth. The body cavity was then opened aseptically and similar cultures were made from body fluid, heart blood, spleen, liver, and kidney. The cultures were incubated for 18 to 20 hr. at 28° C.

Primary cultures from skin lesion scrapings always contained several species of bacteria, including those usually present in normal fish slime. Blood or viscera from recently killed fish usually yielded no viable bacteria or one species alone; from all the tissues of fish that had been out of water for a number of hours, mixed growths consisting of three or four species were frequently obtained. These primary cultures were plated and pure cultures prepared. As far as possible the species were identified. All were tested for pathogenicity in goldfish.

Cultures of *Proteus hydrophilus* were obtained from the 10 diseased pike examined i.e., from the skin lesions of all, from the heart blood of two, from the spleens of two, and from the kidneys of three. Attention was concentrated on this species because of its well known causal relationship to the widespread "red leg" disease of frogs and its known pathogenicity in fish experimentally inoculated.

Pathogenicity

Twenty cultures of *P. hydrophilus* isolated from skin lesions and viscera of 10 pike with "red sore" and from the blood of two frogs with advanced "red leg" have been tested for pathogenicity in goldfish and one was tested for pathogenicity in several other animals.

The cultures, grown for 24 hr. at 28° C. in nutrient broth, were injected intraperitoneally into goldfish in 0.01 cc. doses (0.1 cc. of a 1/10 dilution). Each of the 20 cultures killed the animals in 24 to 72 hr. Doses as low as 0.001 cc. of two of the 24-hr. cultures killed them in five to seven days but doses of less than 0.001 cc. produced no symptoms. In goldfish there was a considerable increase in body fluid. It was slightly viscous, brownish in colour, and contained large numbers of bacteria but there was no increase in cells. Pure cultures of the organisms were obtained from the body fluid, heart blood, and kidneys.

Doses of 0.01 to 0.1 cc. of a 24-hr. culture injected subcutaneously into goldfish caused the development of local necrotic lesions and the fish died in three to five days. The organisms were recovered from heart blood of the dead fish.

An attempt was made to produce infection by light scarification of the skin over an area from which four to five scales had been removed and swabbing the spot with undiluted broth cultures. The fish reacted irregularly; some developed local necrotic lesions in three to five days, others showed no reaction. The addition of 1 to 10 cc. of culture to 2-litre lots of water each containing two goldfish, always failed to produce infection.

Of a 24-hr. culture of *P. hydrophilus* that killed goldfish in doses of 0.01 cc., 0.5 cc. was injected intraperitoneally into a snake, *Thomnophis sirtalis*, which had been in the laboratory for two months. The snake died in four days but there were no gross pathological changes except a considerable increase in blood stained body fluid from which organisms, identical with those injected, were recovered.

The same culture of *P. hydrophilus* was injected subcutaneously into the thigh of frogs in amounts of 0.1 to 0.4 cc. All showed the typical symptoms of "red leg" and died in three to five days. Similar reactions occurred when the infecting organisms were obtained from naturally diseased frogs or pike.

Intraperitoneal injection of 0.1 to 0.5 cc. of the previously mentioned culture of *P. hydrophilus* into four white mice caused death within 18 to 48 hr. No gross lesions were detected but there was an increase in body fluid; it was blood stained but had no marked increase in the number of cells. Organisms, identical with those injected, were recovered from body fluid, liver, and spleen but not from blood.

It is evident that the strains of *P. hydrophilus* recovered from diseased pike are capable of producing fatal infections in goldfish, snakes, and white mice and that in frogs they produce typical "red leg."

Antigenic Structure

Rabbits were immunized with cultures of *P. hydrophilus* recovered from three pike with "red sore" and from one frog with "red leg". This frog was from a lot that had just been received from Quebec. Agglutinating serum of reasonably high titre was obtained in each case; the organisms from each source were agglutinated in serum dilutions of 1/640 and 1/2560. Ten cultures of *P. hydrophilus* recovered from different diseased pike and two from frogs with "red leg" were tested against each of the above sera. The results are summarized in Table I. It is apparent that three cultures from different tissues

TABLE I

AGGLUTINATION REACTIONS OF 10 CULTURES OF *P. hydrophilus*, FROM FOUR PIKE, ONE SUCKER, ONE GOLDFISH, AND ONE FROG, WITH FIVE ANTISERA. FIGURES INDICATE TITRE OF SERUM

Culture	Source	Antisera				
		*A*5–4	*S*1–5	*R*8	*X*2–38	*L*3–2
*A*5–4	Pike *A*	1/640	–	–	–	–
*A*5–3	Pike *A*	1/640	–	–	–	–
*S*1–5	Pike *S*	–	1/2560	–	–	–
*R*8	Pike *R*	–	–	1/2560	–	–
*R*10–3	Pike *R*	–	–	1/2560	–	–
*R*10–1	Pike *R*	–	–	1/2560	–	–
*T*1	Pike *T*	–	–	–	–	–
*U*2	Sucker	–	–	–	–	–
*X*2–38	Goldfish	–	–	–	1/2560	–
*L*3–2	Frog	–	–	–	–	1/640

of pike *R* are antigenically identical as are two from pike *A*. Other cultures are, as far as determined by agglutination, antigenically dissimilar.

This antigenic heterogenicity is in line with what has been observed in other species of the genus *Proteus* by Wenner and Rettger (8) and Taylor (7).

Moltka (5), however, demonstrated that swarming forms of *Proteus vulgaris* could be divided into three groups on the basis of floccular agglutination. It should be noted that the cultures of *P. hydrophilus* at the time of isolation from pike and frogs were in the *H* or swarming form, or, more frequently, in the *O* or non-swarming form. Under the conditions in which they were held in the laboratory, the *H* types rapidly changed to the latter. At the time the rabbits were immunized and the agglutinating antigens were prepared, all the cultures were in the non-swarming form.

Proteus hydrophilus and Its Distribution

Proteus hydrophilus (Chester) Bergey is a small rod 1 to 4 μ long by 0.5 to 1 μ in diameter, occasionally curved or irregular in shape, non-sporulating, and Gram-negative. Individual colonies on nutrient agar are 3 to 5 mm. in diameter, frequently, but not regularly, amoeboid, smooth, translucent, usually stippled, and bluish-white in colour. Acid and gas are regularly formed in glucose, sucrose, maltose, and mannite. Lactose, xylose, salicin and inulin are not fermented. The methyl red reaction is positive. Hydrogen sulphide is formed, gelatine is rapidly liquefied, much indol is formed. The organism was first isolated by Sanarelli (6) in 1891, and described in detail by Emerson and Noris (2) and Klup and Lackman (4) as the causal organism of "red leg" in frogs. It is probable that insufficient work has been done on this species to definitely establish its systematic position. In several studies of the genus *Proteus*, particularly that of Wenner and Rettger (8), it is not mentioned.

Recently Fish (3) has isolated *P. hydrophilus* from "ulcer disease" in fingerling brook, rainbow, blackspotted, and lake trout in the Cortland, New York, hatchery. This description of "ulcer disease" as well as an earlier description by Calkins (1) indicates that it is very similar if not identical with what has been found in the pike. The principal difference appears to be that the infected areas in the pike are much reddened, whereas Fish, found the lesions in trout to vary from a grey-white induration of the skin to a deep red necrotic ulcer. This difference may be the reaction in a different species of fish or possibly to the fact that an earlier stage of the disease has been observed in the hatchery. There are no trout in the regions in which the disease in pike has been studied.

Fish's observation, together with a private communication from Dr. R. H. M'Gonigle of the Atlantic Biological Station, suggests that infections with this organism may be an important factor in rearing of fish in the hatchery.

Acknowledgment

The writers are indebted to the National Committee on fish culture for financial assistance.

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A NEW METHOD FOR WASHING PARAMECIA[1]

By F. T. Rosser[2]

Abstract

The freeing of paramecia from bacterial contamination by passage through five one-tenth millilitre drops of sterile media is demonstrated. Wash drops are pipetted into the centre of Petri dishes. Paramecia are transferred from drop to drop in a small loop of 0.127 mm. platinum wire. They are allowed to remain for at least 15 min. in each wash, with the exception of the third in which they are kept for about 4 hr. Animals can be seen without difficulty when light is focused on the drop in which they are swimming. Addition of vitamins in various concentrations to the medium and association of a number of sterile paramecia in one tube failed to promote divisions.

Introduction

The method of freeing paramecia from bacteria as described by Parpart (1) and modified by Kidder *et al.* (2) is a procedure involving considerable time and an appreciable amount of equipment. The object in undertaking the present study was to develop a simpler washing technique.

In the scheme followed a platinum wire loop sterilized by flaming was used to transfer paramecia through a series of sterile wash fluid drops (1/10 ml.) contained in Petri dishes. This procedure eliminated wash fluid containers and sterile capillary pipettes. Since the work could be done at a transfer table wiped over with a disinfectant, a hood was unnecessary. Furthermore, by improvement of the washing technique, half the number of transfers sufficed to obtain bacteria-free paramecia.

Methods

In practice the one-tenth millilitre drops of wash fluid were placed in sterile Petri dishes with a pipette. No serious difficulty was experienced by drops of this size spreading. The loop (diameter 1 mm.) was twisted at the end of a 2-in. length of 30 gauge platinum wire inserted in a bacteriological needle holder. To facilitate operations it was bent off at right angles to the straight wire which was bent again at right angles about one-half inch from the loop, thus forming a Z at the end of the wire. It was found convenient to work on the glass stage of a dissecting microscope from which the tube was removed, since light could be focused on the drop making the paramecia easily visible to the naked eye. By brushing the loop over or lifting it up under an individual, single animals were readily isolated and held in the film formed in the loop. The paramecium was released when the loop was touched to a wash drop allowing the animal to swim away. Successive transfers were accomplished in a similar manner.

[1] *Manuscript received March 15, 1941.*

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as N.R.C. No. 988.

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The animals frequently swam into the twists of wire at the base of the loop making it difficult to transfer them. This trouble was overcome by using a fine wire (diameter 0.127 mm.) free from twists, with the loop diameter somewhat less than 1 mm. and the end of the wire just touching at the first 90° bend. This loop not only facilitated transfer but carried less of the contaminated fluid to the next drop.

By extending the time in each wash it was possible to reduce the required number of washings from ten or more to five. The procedure finally adopted was to place a number of individual animals in a corresponding number of individual drops. When the first animal had been washed for at least 15 min. the operation was repeated transferring all the animals, in the same sequence, to the next wash drop. In this way 18 or 20 paramecia could readily be transferred from one wash to another in less than half an hour. Animals were allowed to remain 4 hr. in the third wash. To prevent evaporation about 1 ml. of sterile wash fluid was run out around the edge of these plates.

Pringsheim's medium for *P. bursaria*, to which was added 0.5% Difco proteose-peptone as recommended by Loefer (3), was used for washing and culturing. As described later, various concentrations of vitamins were added to the culture medium to determine their effect on promoting divisions.

To test the efficacy of the technique, wash plates were poured with Difco beef agar as soon as possible after removing the animal and were incubated at 28° C. for three days before reading. Tube cultures of washed paramecia were incubated at 20° C. for six days following which 1 ml. of the culture medium from each tube was plated to determine bacterial contamination.

Washing Experiments

In the first experiment the effectiveness of the loop transfer method in freeing paramecia of bacteria was tested. The animals used were from a hay infusion culture inoculated seven days before with a few millilitres of pond water swarming with paramecia. Table I shows the results of washing 10 paramecia. They were allowed to remain about 2 min. in each wash drop with the exception of the fifth in which they were kept 5 hr. Each one was transferred from its tenth washing to a culture tube.

TABLE I

EFFECTIVENESS OF THE LOOP TRANSFER METHOD IN FREEING PARAMECIA OF BACTERIA

Number of paramecia washed	Wash No.										Cultures after 6 days at 20° C.
	1	2	3	4	5	6	7	8	9	10	
	Average number of bacteria per wash										
10	10,000	266	31	3	33	1	0.6	0.6	0.4	0.3	Sterile

Since the average bacterial count after the fifth washing was one or less, and the colonies that appeared could be regarded as air contaminants (the plate covers having been raised four times), it was concluded that the number of washings necessary to obtain freedom from bacteria could be reduced.

A second experiment was undertaken to determine whether bacteria could be eliminated by five washings and whether the 5-hr. decontamination period should be made in conjunction with the third or fourth washing. The paramecia used were from a pure line strain isolated from pond water two months previously and grown in hay infusion medium.

TABLE II

EFFECTIVENESS OF FIVE WASHINGS IN FREEING PARAMECIA OF BACTERIA

Paramecium No.	Held five hours in wash No. 3						Held five hours in wash No. 4					
	Wash No.					Cultures after 6 days at 20° C.	Wash No.					Cultures after 6 days at 20° C.
	1	2	3	4	5		1	2	3	4	5	
	Number of bacteria per wash						Number of bacteria per wash					
1	2300	400	40	0	0	Sterile	1000	24	1	35	0	Contaminated
2	600	275	18	0	2	Sterile	400	87	1	47	0	Contaminated
3	1600	21	24	0	0	Sterile	500	135	7	47	0	Sterile
4	1200	2	15	1	2	Sterile	600	0	0	39	1	Sterile
5	700	146	65	0	0	Sterile	1500	2	0	64	0	Contaminated
6	1000	56	56	0	0	Sterile	1700	340	8	49	0	Sterile
7	1000	2	115	0	0	Sterile	400	0	1	44	0	Contaminated
8	1100	98	80	0	0	Sterile	1200	2	3	74	0	Contaminated
9	600	3	78	0	0	Contaminated	1900	200	6	41	1	Contaminated
10	2000	10	34	0	0	Contaminated	1700	220	3	44	0	Sterile
11	900	475	68	0	1	Sterile	900	48	0	112	1	Sterile
12	2200	2	36	3	2	Sterile	700	0	0	13	0	Contaminated
13	600	36	72	0	0	Sterile	800	47	1	24	0	Sterile
14	400	0	58	0	0	Sterile	600	1	1	144	0	Sterile
15	1400	1	46	0	0	Sterile	500	425	4	29	2	Contaminated
16	1200	250	118	15	0	Sterile	700	53	0	30	0	Sterile
17	300	390	82	0	0	Sterile	700	57	1	90	1	Sterile
18	500	400	33	0	0	Sterile	500	0	0	30	0	Sterile
Total	19,600	2567	1038	19	7		16,300	1641	37	956	6	
Average	1089	143	58	1	0.4		906	91	2	53	0.3	

NOTE: *Number of bacteria in parent culture: 52,000,000 per cc.*

Table II shows that the best results were obtained when the decontamination period accompanied the third washing. The fact that the paramecium sometimes became trapped in the twisted wire at the base of the loop, necessitating a transfer of the entire drop, probably accounts for most of the variations in bacterial numbers. When the animal remained in the loop, transfer was accomplished with a minimum carryover of medium. It is obvious that numbers of adhering bacteria were shed in the first three washings, and since eight of the ten contaminated tube cultures were from paramecia

held 5 hr. in Wash 4 (the organisms in nine of the tubes appeared to be coccus forms), it seems clear that sufficient time had not been allowed in the individual washings for the animals to free themselves from adhering bacteria.

The third series of experiments was designed to compare the number of bacteria transferred in the medium with the number transferred by the animal. Single paramecia were transferred by touching the loop to sterile drops. Similarly a loopful of parent medium without a paramecium was just touched to a second drop and a third loopful was thoroughly mixed with another drop. The effect of a thin film of oil on the plates was also determined. The improved loop and washing procedure previously described were used throughout. The paramecia were from a 13-day old culture of the strain used before.

TABLE III

EFFECTIVENESS OF LOOP TRANSFER METHOD IN REDUCING NUMBER OF BACTERIA TRANSFERRED IN THE CULTURE MEDIUM

Paramecium No.	Plates oiled								Plates not oiled				
	Number of bacteria per loop	Number of bacteria transferred by touching loop to drop	Wash No. 1	Wash No. 2	Wash No. 3	Wash No. 4	Wash No. 5	Cultures after 6 days at 20° C.	Wash No. 1	Wash No. 2	Wash No. 3	Wash No. 4	Wash No. 5
			Number of bacteria per wash						Number of bacteria per wash				
1	2000	600	500	2	21	3	1	Sterile	180	14	2	1	1
2	1400	900	800	1	15	0	0	Sterile	270	13	2	0	0
3	2900	1000	1000	5	7	0	0	Sterile	400	5	2	0	0
4	2600	1200	1500	16	3	0	0	Sterile	320	14	4	0	0
5	2400	1000	1300	3	1	2	0	Sterile	420	14	5	0	0
6	1900	1200	1300	14	5	0	0	Sterile	400	7	3	0	0
7	2600	1300	800	2	3	0	0	Sterile	480	55	6	1	0
8	2700	1600	1000	23	8	0	0	Sterile	280	0	6	0	0
9	2800	1000	900	9	5	0	0	Sterile	400	4	2	1	1
10	1700	1800	1600	5	3	0	0	Sterile	320	3	9	0	0
11	2900	500	1200	2	2	0	0	Sterile	250	8	3	0	0
12	1800	900	1500	1	3	1	0	Sterile	170	1	11	0	0
13	2000	1700	1100	7	0	0	0	Sterile	460	4	1	0	0
14	3000	1200	1900	5	5	0	0	Sterile	270	14	4	0	0
15	2100	500	1700	4	18	0	0	Sterile	250	4	2	0	0
16	1600	800	1500	8	9	0	0	Sterile	300	0	3	0	0
17	2500	1400	1600	14	0	0	0	Sterile	90	8	2	0	0
18	2100	700	1300	4	33	0	0	Sterile	440	2	2	0	0
Total	41,000	19,300	22,500	125	141	6	1		5700	170	69	3	2
Average	2278	1072	1250	7	8	0.3	0.05		317	9	4	0.2	0.1

NOTE: *(a) Number of bacteria in parent culture: 26,500,000 per cc.*
(b) Tube culture of 18 paramecia from second series after 6 days at 20° C. was sterile.

Results in Table III show that only about half as many bacteria were transferred by touching the loop to a sterile drop as by transferring the entire contents of the loop. The additional number of bacteria carried over by the

transfer of an animal was comparatively small and most of these were left behind in the first wash. The culture medium itself was the largest contributor of bacterial contamination. The count can be reduced by about 1/10,000 per transfer by the loop method, near sterility of the medium being reached at the second transfer. Once this is obtained it is only necessary to allow sufficient time in succeeding washes for the animals to free themselves from closely adhering or ingested bacteria. The indications are that by further adjustment of the washing period, freedom from bacteria may be reached in still fewer washings.

The bacterial numbers observed in the first washes from oiled and unoiled plates show that about four times as many bacteria were transferred in the former. This indicates that more of the liquid medium was carried across when oil was present. Oil bubble reflections made it difficult to locate paramecia in the wash drops and to read the number of bacterial colonies on the plates. Oil had little effect on preventing the spread of drops.

Some Preliminary Growth Experiments

In the first experiment none of the paramecia lived for longer than seven days and no divisions occurred. It was found in a previous study that the majority of single isolates lived only a few days and failed to divide regardless of the bacterial content of the medium. Yet hay infusion flasks sowed with a few millilitres from a heavy paramecium culture invariably provided a new heavy culture in the course of five or six days. These results suggested that perhaps the medium lacked a growth promoting substance produced in sufficient quantity only when a large number of animals were present. To test this supposition paramecia were cultured in duplicate tubes of Pringsheim's medium containing 1 and 10 parts per million of the following substances: ascorbic acid, calcium pantothenate, nicotinic acid, oestriol, riboflavin, and inositol. Saturated and 1/10 saturated aqueous solutions of thyroxine were also used. In addition quadruplicate tubes were included combining all these substances in both the high and low concentrations. Since there was some indication that thyroxine might affect division, triplicate tubes of medium containing saturated, 1/4, 1/16, and 1/64 saturated aqueous solutions of thyroxine were also tried.

With the exception of two media (containing saturated and 1/10 saturated thyroxine) in which one division took place, no divisions occurred in any tube. No marked differences between treatments were noticed with the exception that 10 parts per million of riboflavin was harmful. The animals receiving these treatments and the higher concentration combination treatments died within one day. A few animals were dead each day. Ten of the 72 included in these tests were still living when the cultures were tested for bacteria on the sixth day.

Eighteen paramecia washed in the last series of experiments were put into one tube to determine if the association of a number of bacteria-free animals would have any effect on their reproduction. No divisions were observed and the death rate was similar to those placed in separate tubes.

Acknowledgment

The author wishes to acknowledge the assistance of Mr. A. M. Adams, Laboratory Assistant, National Research Laboratories.

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ON THE FOOD OF SEALS IN THE CANADIAN EASTERN ARCTIC[1]

By M. J. Dunbar[2]

Abstract

The stomach contents of 47 ringed seals (*Phoca hispida*), five bearded seals (*Erignathus barbatus barbatus*), and one harp seal (*Phoca groenlandica*), from Baffin Island waters, are described.

It is shown that *Phoca hispida*, during the months of August and September at least, is predominantly a plankton-eater, the bulk of its food consisting of the pelagic amphipod *Themisto libellula*, and to a much lesser extent, the schizopod *Mysis oculata*. The stomach of the harp seal also contained planktonic species only.

The food habits of the ringed seal are discussed in relation to the plankton succession in the fjord waters.

Introduction

Stephensen (11) records the finding of large numbers of the planktonic euphausid, *Thysanoessa inermis*, in the stomachs of harp seals (*Phoca groenlandica*), in east Greenland, and comments on the novelty of this occurrence: "As far as I know we have here for the first time euphausids found in great multitudes in the stomachs of seal" and "Sometimes crustacea have been found in the stomachs of seals, but the records previously known were few, and the finds seem to be quite accidental." This is not strictly true. Andersson (1), Barrett-Hamilton (2), and Trouessart (13), all record that the Antarctic crab-eater seal feeds almost exclusively on planktonic crustacea, mainly *Euphausia superba*; this has also been noted recently by Bertram (3) and Lindsey (8). Barrett-Hamilton (2) found Weddell's seal, another Antarctic species, feeding on *Euphausia* in the pack ice, a fact that is not corroborated by other workers, though Lindsey (9) found the very young of this species feeding on *Euphausia*. Of the workers in the Arctic region, Johansen (6) found the stomachs of all the seals (*Phoca hispida*) shot in the drift ice during June and July full of pelagic amphipods, whereas Kumlien (7) and Hantzsch (5) found *Phoca hispida* feeding largely on crustacea of an undefined type. More recently Sutton and Hamilton (12) said of *Phoca hispida* taken in May at Southampton Island: "The stomachs we had opportunity to examine were in every case well filled with the remains of "Kingook", an abundant crustacean, and with small fish." (The Eskimo word" Kingook" is in point of fact applied to almost any small crustacean of an amphipod-like shape.)

The present paper describes material that shows that the bulk of the food of the jar seal, or ringed seal, *Phoca hispida* Schreber, in the waters of the Baffin Island coasts, consists of planktonic crustacea, at least in the summer months. Seals with such plankton-eating habits are apparently not found

[1] *Manuscript received for publication February 19, 1941.*
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in more temperate regions, where the plankton is neither so large nor so abundant. In view of the bad reputation of seals amongst fishermen, it is interesting to find how few seals are consistent fish-eaters.

Material

As a member of the Canadian eastern Arctic Patrols of 1939 and 1940, and while making a study of the marine plankton of the Baffin Island coasts, Hudson Strait, and northern Labrador, the author examined the stomachs of 47 ringed seals, five bearded seals (*Erignathus barbatus barbatus* Erxleben), and one harp seal (*Phoca groenlandica* Erxleben). The bearded seal is a strictly benthonic feeder, eating bottom-living crustacea, mollusca, and fish. The contents of the five stomachs examined consisted of the following:

Sclerocrangon boreas (Phipps)
Hippolyte (*Spirontocaris*) *polaris* (Sabine)
Hippolyte (*Spirontocaris*) *spinus* (Sowerby)
Remains of sculpin, probably *Myoxocephalus groenlandicus* (Cuvier and Valenciennes)
One tubicolous worm, damaged and not identifiable.

The stomach of the harp seal, examined on August 8, 1940, at Frobisher Bay, was full of *Mysis oculata* (Fabr.) in large numbers, together with four

TABLE I

STOMACH CONTENTS OF *Phoca hispida*, 1939. SPECIES IN BOLD-FACE TYPE REPRESENT BULK OF CONTENTS

Date	Station	Number of seals	Stomach contents
Aug. 2	Lake Harbour	1	***Themisto libellula*** — Full
Aug. 4	Lake Harbour	2	***Themisto libellula*** — Full
Aug. 4	Lake Harbour	6	***Themisto libellula*** — Five full, one half full
Aug. 7	Lake Harbour	11	***Themisto libellula*** — Five full, five half full, one almost empty
			Also, in these 20 stomachs:
			Hyperia spinigera 1 specimen
			Pseudalibrotus nanseni glacialis 2
			Gammarus locusta sp. 8
			Gammaracanthus loricatus 5
			Meganyctiphanes norvegica 1
			Thysanoessa raschii 1
			Mysis oculata 1
			Mictheimysis mixta 14
			Vertebral column and muscle of small fish not identifiable 1
Aug. 7	Lake Harbour	2	Empty
Aug. 7	Lake Harbour	1	Liquid only.

TABLE II

STOMACH CONTENTS OF *Phoca hispida*, 1940. SPECIES IN BOLD-FACE TYPE REPRESENT THE BULK OF THE CONTENTS

Date	Station	Number of seals	Stomach contents	
Aug. 4	Gabriel Strait S.E. Baffin Is.	1	***Mysis oculata*** *Themisto libellula*	Full 4 specimens
Aug. 13	Lake Harbour	1	***Themisto libellula***	Full
Aug. 13	Lake Harbour	1	Empty	
Aug. 20	Lake Harbour	2	Empty	
Aug. 20	Lake Harbour	5	***Themisto libellula*** *Thysanoessa raschii*	Full 1 specimen
Aug. 20	Lake Harbour	1	***Mysis oculata*** *Themisto libellula* *Hippolyte ?gaimardi*	Full 2 specimens 1
Aug. 22	Lake Harbour	4	***Themisto libellula***	Full
Aug. 22	Lake Harbour	1	***Themisto libellula***	Half full
Sept. 9	Clyde River N.E. Baffin Is.	2	Almost empty, contained altogether: *Themisto libellula* *Mysis oculata* *Mictheimysis mixta* *?Myoxocephalus groenlandicus*	1 specimen 18 3 2
Sept. 14	Clyde River	1		
Sept. 17	Clyde River	1		
Sept. 13	Clyde River	1	Empty	
Sept. 14	Clyde River	1		
Sept. 17	Clyde River	1		
Sept. 17	Clyde River	1	About one-third full; contained: *Calanus finmarchicus* *Metridia longa* *Themisto libellula* *Apherusa glacialis* *Gammarus locusta* *Thysanoessa inermis* *Mysis oculata* *Mictheimysis mixta* Decapod larvae *Limacina helcina* *?Cyclogaster fabricii* Scraps of small fish, very little	1 specimen 2 1 2 5 1 33 1 4 142 1

specimens of *Mysis mixta* Lilljeborg, and 22 specimens of *Themisto libellula* (Mandt). *Mysis oculata*, although usually found near the bottom in shallow water, must be considered a planktonic species. It is caught in the tow-net rather than in the dredge.

The contents of the stomachs of *Phoca hispida* are given in Tables I and II. Species in bold-face type represent the greater proportion of the contents. The complete list of animals found in *Phoca hispida* is as follows:

Calanus finmarchicus (Gunnerus)
Metridia longa (Lubbock)
Hyperia spinigera Bovallius

Themisto libellula (Mandt)
Pseudalibrotus nanseni G. O. Sars
Pseudalibrotus glacialis G. O. Sars
Apherusa glacialis (H. J. Hansen)
Gammarus locusta (Linné)
Gammarus sp.
Gammaracanthus loricatus Lovén
Meganyctiphanes norvegica (M. Sars)
Thysanoessa raschii (M. Sars)
Thysanoessa inermis (Kröyer)
Mysis oculata (Fabr.)
Mysis mixta Lillj. (= *Mictheimysis mixta* (Lillj.))
Decapod larvae
Hippolyte (*Spirontocaris*) ?*gaimardi* Milne-Edwards
Limacina helicina (Phipps)
? *Cyclogaster fabricii* (Kröyer)
? *Myoxocephalus groenlandicus* (Cuvier and Valenciennes)

Results and Discussion

It is apparent from Tables I and II and from the species list that *Phoca hispida*, at least during the months of August and September, is predominantly a planktonic feeder in Baffin Island waters. The work of Kumlien (7), Sutton and Hamilton (12), and Johansen (6) extends the time-scale of this habit back to May, and includes Southampton Island and northeast Greenland in its geographical range.

There are other points of interest. The seals examined in August, 1939 and 1940, in southern Baffin Island (Lake Harbour and Frobisher Bay) were, with very few exceptions, well fed, and at that time there was an abundance of *Themisto libellula* in the plankton. Towards the latter half of August, and in September, *Themisto* was scarce in the plankton of the coastal water, both at Lake Harbour and in the north, at Clyde River, and the seals at Clyde River, taken in September, were virtually starving. It is apparently not possible for them to make up their diet with fish; nor, it seems, can they dig for bottom-living crustacea and mollusca as the larger *Erignathus* is able to do; other planktonic species are either not of sufficient size or in sufficient numbers to make up for the lack of *Themisto*. The result is that the ringed seal must go through a lean time in the late summer. Anything available is eaten—it is interesting that one seal was found eating the small planktonic mollusc *Limacina helicina*, a species that appears in enormous numbers at that time, following on the *Themisto* population.

The fate of the *Themisto* population is not relevant to this paper and will be discussed elsewhere; but with the disappearance of *Themisto* from the fjord waters during the summer months, there is an accompanying scarcity of seals. According to information from residents in the country, both White and Eskimo, they return late in September or in October, and it seems that in winter

and spring the ringed seal is still eating "small crustacea". (Kumlien (7), however, found them subsisting mainly on fish in Cumberland Gulf during the winter.) The occurrence of ringed seal, perhaps, follows the greatest abundance of the larger planktonic crustacea, notably *Themisto libellula* in the Baffin Island area, and it is possible that the starving seals examined at Clyde River were stragglers who failed to follow the majority.

There is, however, another possible explanation of the behaviour. The ringed seal is not simply an aquatic mammal; it is an animal whose habits are closely associated with the ice, and it seems more probable that the majority leave the fjord waters with the ice, in the early summer (July), and that those left behind are responsible for the drastic reduction of the numbers of *Themisto libellula* as shown by the results of the tow-netting operations in August. Once the *Themisto* population is depleted so as to be no longer a food supply, the seals left in the fjords become scavengers, mainly on the plankton.

In this connection, information is plainly needed concerning the following points:

1. The plankton populations in the neighbourhood of the ice in the open water of Hudson Strait and Baffin Bay.
2. The food habits of the seal in these open waters in August and September.
3. The nature of the plankton, and the food of the seals, at the time of freeze-up in the fall.

Two of the seals, those caught on August 4 and August 20, 1940, had been eating *Mysis oculata*, not *Themisto libellula*. One of these was from Gabriel Strait, and the tow-netting operations carried out there confirmed the inference from the stomach of the seal, that *Themisto* was scarce in the locality. The other came from near Lake Harbour, and was shot by an Eskimo not more than 20 miles from the settlement. Five other seals brought in on the same day had their stomachs full of *Themisto*, as did those examined on August 22, two days later (Table II). It may be inferred from this that the distribution of one of these species is patchy, according to which one is preferred by the seals; it seems to be *T. libellula*. In either case, whether the seal is eating *Mysis* or *Themisto*, the method of feeding is the same, i.e., planktonic.

There appears to be a significant difference in the manner of feeding of the two plankton-eating seals, *Phoca hispida* in the Arctic and *Lobodon carcinophagus*, the crab-eater seal, in the Antarctic, in that the former is selective and the latter is not. Barrett-Hamilton (2) quotes M. Racovitza on Lobodon as follows: "Il nage la bouche ouverte dans les bancs de ces crustacés, (*Euphausia superba*), à la façon des baleines, et en consomme de grandes quantités." And he adds: "I think it very probable that some light is thus thrown upon the wearing and use of the extraordinarily complicated cheek-teeth, the cusps of which may form a sieve through which is strained the water taken into the mouth with the euphausids." No such behaviour of *Phoca hispida* has ever been recorded. Furthermore, during the summer in Baffin

Island waters the *Themisto* show a strongly bimodal size distribution, similar to, but more strongly marked than, the size distributions described for other Arctic planktonic animals from Greenland by Stephensen (10) and Dunbar (4). Only the larger-size group of *Themisto* was found in the seal stomachs, and it may be assumed that the ringed seal takes only the larger specimens. The dentition of *Phoca hispida* is not of the *Lobodon* type, and indeed it is remarkable that an animal that feeds so much on small crustacea should have such sharp and efficient teeth. The crustacea are almost intact, for the most part, when they are found in the stomach.

Parasitic worms were plentiful in the stomachs of the bearded seal, and also in the single specimen of the harp seal, but *Phoca hispida* was comparatively free of them. Seals examined in 1939 showed none at all, and in 1940 only three individuals possessed parasites, in small numbers. All three were taken at Clyde River, and were starving. Bertram (3) found, similarly, that the plankton-eating *Lobodon* of the Antarctic "rarely harboured a single species" of gut parasite, whereas the fish-eating Weddell's seal was full of them. It would seem that the parasites are derived from the food eaten.

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